Nutrient Pollution of Streams in the Illinois River Watershed, Oklahoma: Effects on Water Quality, Aesthetics, and Biodiversity

Expert Report of Dr. R. Jan Stevenson

For

State of Oklahoma

In

Case No. 05-CU-329-GKF-SAJ

State of Oklahoma v. Tyson Foods, et al.

(In the United States District Court for the Northern District of Oklahoma)

R. Jan Stevenson

Dr. R. Jan Stevenson
Professor of Zoology
Aquatic Ecologist

EXHIBIT R

Contents

EXHIBIT R

EXHIBIT R

Section 1
Background

1.1 Objectives

This report reviews knowledge about nutrient pollution of streams and recent findings on causes and effects (injuries) related specifically to nutrient pollution in streams of the Illinois River Watershed (IRW).

Nutrient pollution of streams, particularly with phosphorus (P), causes degradation of water quality, violates narrative and numerical water quality standards, and is a substantial cause of injury to beneficial uses, such as aesthetics and fish and wildlife propagation. The application of poultry waste to lands surrounding poultry houses is a substantial source of P in streams of the IRW (Expert Witness Report of Dr. Bernard Engel). The watershed of Lake Tenkiller, including the Illinois River, Flint Creek, and Baron Fork Creek and their tributaries, is designated as a Nutrient Limited Watershed due to the adverse effects of excess nutrients on a designated beneficial use (OAC 785:45-5-29). Scenic rivers located in the IRW are known to exceed the P criteria (0.037 mg/L) established by the State of Oklahoma (OWRB 2005). The recent findings in this report are based on extensive studies of IRW streams that were designed to evaluate and document the resulting harm/injury to natural systems that has resulted from the disposal of poultry wastes within the IRW.

An overview of what is known about effects of nutrient pollution on aesthetics and species composition of biota in streams is described in the Background Section, along with definitions of terms. In subsequent sections of this report, I describe the results of field studies in which we show:

- High nutrient concentrations in streams of the IRW;
- Significant relationships between P concentrations and poultry house density;
- Significant and substantial direct and indirect effects of poultry house density and nutrients on algal biomass, dissolved oxygen (DO), and pH; and
- Significant and substantial direct and indirect effects of the above casual factors (poultry house density, P, algal biomass, DO, and pH) on species composition of algae, invertebrates, and fish.

Throughout the report there is extensive reference to similar findings in other studies that have been published in the peer-reviewed scientific literature.

1.2 Nutrient Pollution in Streams

1.2.1 Stream Ecology

Stream and lake ecosystems differ primarily because streams are shallower and have unidirectional flow. Because streams are shallow, most biological activity is associated with the bottom of the stream. Downstream transport of nutrients supports the growth of algae on the

EXHIBIT R

bottom of the stream, which is referred to as benthic algae. Benthic means attached to or associated with the bottom of aquatic ecosystems. Many benthic invertebrates graze on the algae as a food source, and others utilize decomposing leaves and organic material that has fallen into the stream from terrestrial sources. Some fish are herbivorous, particularly the stoneroller which has a cartilaginous ridge in its lower jaw for scraping algae from rocks. Most fish are either omnivorous (eating many kinds of food) or predaceous (eating invertebrates or other fish).

The size, permanence, and food sources in streams change from headwater streams to large rivers, which affect the kinds of organisms that live in them (Vannote et al. 1980). Headwater streams are narrower, often covered by a canopy of trees, and more likely to dry out during summer than larger rivers. The shading by trees reduces the relative importance of algae as a food source in headwater streams and increases importance of terrestrial litter. Reduced or non-existent stream flow during summer constrains species composition to organisms that can recolonize from upstream or downstream refugia, survive drying, or have short generation times. Midsize streams often have more algae than small streams because the tree canopy separates over the channel as streams get wider. Invertebrates adapted to eating algae versus leaf litter are more important in midsize than headwater streams. Species composition of fish also changes with food sources and permanence as streams increase in size.

Many kinds of algae grow in streams. The most common algae in streams, and perhaps in all waters around the world, are diatoms. Diatoms are unicellular algae with glass cell walls (**Figure 1.1A**). They appear golden brown in streams because the dominant photosynthetic pigment in them is golden brown, rather than green as in plants (**Figure 1.1B**). Filamentous green algae (FGA) are also important in streams, such as the genus *Cladophora* (**Figure 1.1C, D**). Many FGA do not grow in low nutrients; when their abundance is great, they can alter the habitat physically and chemically in ways that affect the other organisms in the stream (Dudley et al. 1986, Parr and Mason 2004). Cyanobacteria (also called blue-green algae) are the third major kind of algae that grows abundantly in streams (**Figure 1.1E**). These algae are poor food sources compared to diatoms (Fuller and Fry 1991). As streams increase in size to rivers, and water retention increases, algae suspended in the water grow (see review by Stevenson in press). These are called planktonic algae or phytoplankton. Blooms of planktonic cyanobacteria in nutrient-rich waters are aesthetic problems, but may also threaten human health (Pearl and Huisman 2008).

Insect larvae are the most diverse and abundant animals in many streams. The common orders of aquatic insects are the Ephemeroptera (mayflies), Plecoptera (stoneflies), Trichoptera (caddisflies), and Diptera (true flies) (**Figure 1.2**). The first three orders tend to be more sensitive to pollution than the latter (Hilenshoff 1988). This sensitivity to pollution is partly due to adaptations to breathing. Mayfiles and stoneflies have gills (**Figure 1.2A, B**), whereas some diptera have breathing tubes and other adaptations for low oxygen waters (Merritt and Cummins 2007). Most species of mayflies are either grazers (eating benthic algae) or collector-gathers, which gather fine particulate matter from the stream bottom, which is a mixture of decomposing litter, bacteria, and algae. Stoneflies are also collector-gathers as well as predators and shredders. Shredders break up large leaf litter. Caddisfly species are usually grazers, collector-gathers, or filter-feeders. Caddisflies building stone cases are protected from predators as they, like snails, graze algae from the surface of rocks (Figure 1.2). Net-spinning caddisflies secrete threads to create webs that filter suspended particles floating downstream (Figure 1.2). The feeding preferences are referred to as

EXHIBIT R

the functional feeding groups of invertebrates, and they vary from species to species. In addition to insect larvae, the other common small animals in streams are snails, clams, and worms.

Fish in streams range in size and habitat from the small minnows and darters occupying margins of streams and small spaces between rocks to the suckers, bass, bullheads, and catfish living in deeper sections of the stream. The lack of year-round flowing water in small streams is one of the major factors constraining species composition of fish in headwater streams (Fritz, et al. 2002, Capone and Kushlan 1991, Scholsser 1982). The diversity of fish increases greatly from headwaters to large rivers. This change in fish diversity and species composition, as well as their motility, makes it difficult to observe environmental impacts on fish. Fish in streams are particularly sensitive to changes in oxygen dissolved in the water, because they require oxygen for respiration and will typically move from areas of low DO to higher concentrations, if available.

1.2.2 Nutrient effects on stream ecosystems

Contamination of streams, lakes, wetlands, and coastal waters of the world with nutrients is one of the most important environmental problems around the world (Carpenter et al. 1998, Smith et al. 1999, USEPA 2007). Low concentrations of nutrients occur naturally in water and are essential for the growth of algae, which supports the production of invertebrates and fish. Nutrient pollution, the loading of nutrients from human activities in streams and rivers, causes many instream as well as downstream problems. The intensity of those problems increases with increasing amounts of nutrient pollution. This problem has become so important in the United States (US) that during the later part of the Clinton Administration, the United States Environmental Protection Agency (USEPA) implemented the Clean Water Action Plan. This plan called for all states and tribes to establish nutrient criteria for all waterbodies to protect the uses of those waterbodies.

Recreation and aquatic life use are two valued ecological attributes, more recently called ecosystem services (MEA, 2005). Recreation by fishing, canoeing, rafting, and swimming in the streams and rivers is an important use of the water. Excess algae in the water can reduce the desirability of these activities (**Figure 1.3**). A survey by the Montana Department of Environmental Quality (Tepley 2007, [illegible]) showed that desirability of recreational use of waters declined precipitously when algae on the stream bottom exceed a specific level, 150 mg/m^2 of chlorophyll a (chl a). The mg chl a/m^2 is a measure of the pigments (chlorophyll) in algae over an area (meter squared – m^2) of stream bottom. Aquatic life use, also called biological condition (Davies and Jackson 2006), is also a use that is protected by the Clean Water Act. States are required to regulate waters to protect aquatic life use and the "biological integrity" or "support of fish, shellfish, and wildlife" of aquatic ecosystems. In addition to protecting the biodiversity of our waters, the goal of this protection is to protect other uses of our water for which the natural functioning of aquatic ecosystems is important, as well as human health (e.g. drinking water).

Throughout this report, several terms are generally used to refer to species composition and biodiversity of organisms in streams and their linkage to state and federal water quality standards and regulations. Let me briefly provide an overview of these terms.

EXHIBIT R

- *Species composition refers rather specifically to the list of species and abundances of each species in a habitat.*

- *Biodiversity is a broader term, referring to species composition as well as the number of species. It can also refer to the genetic diversity and landscape diversity in a region.*

- *Biological condition, reference condition, biological integrity, and aquatic life use have regulatory significance.*

- *Biological condition is the similarity of species composition, biomass, productivity, and other biological processes to natural condition. Measures of deviations in these biological attributes from natural or best attainable conditions have been used to characterize biological condition (Davies and Jackson 2006, Stoddard et al. 2006).*

- *Reference condition is the physical, chemical, and biological condition found in streams having watersheds with the lowest level of human activities.*

- *Biological integrity is the highest level of biological condition attainable in a region. It varies among regions because of regional alterations of landscapes by human activities. Thus the definition of biological integrity varies among regions as best attainable reference conditions vary among regions (Stoddard et al. 2006). However, the definition is anchored in the linkage to natural conditions, defined as having minimal evidence of human effects (Davies and Jackson 2006).*

- *The State of Oklahoma has established the policy that all waters of the state be protected from degradation in accordance with the antidegradation policy set forth in OAC 785:45-3-1. Water quality standards establish a specific antidegradation for scenic rivers and their tributaries that provide "[n]o degradation of water quality shall be allowed in these waters" (OAC 785:45-3-2).Antidegradation standards also prohibit water quality degradation that will interfere with attainment or maintenance of any beneficial use of water (OAC 785:45-3-2). For scenic rivers, the antidegradation standards also require that the thirty day geometric mean concentration of total phosphorus not exceed 0.037 mg/L (OAC 785:45-5-25).*

- *Beneficial uses are designated for all waters of the state and are protected through the antidegradation policy narrative criteria and numerical water quality standards (OAC 785:45-5-2). Beneficial uses for the Illinois River and its tributaries include among others aesthetics and fish and wildlife propagation (OAC 785:45-5-3 and Appendix A). For example, the 0.037 mg/L total phosphorus criterion for scenic rivers in Oklahoma protects an aesthetics beneficial use (OAC 785:45-5-19). In addition to this criterion, narrative criteria for the protection of the aesthetics beneficial use require the water to be free from floating material and suspended substances that produce objectionable color, materials that settle to form objectionable deposits and discharges that produce undesirable effects or are a nuisance to aquatic life (OAC 785:45-5-19). Narrative criteria also provide that nutrients shall not cause excessive growth of periphyton, phytoplankton, or aquatic macrophyte communities which impairs any existing or designated beneficial use (OAC 785:45-5-9). Narrative criteria further require that the surface waters of the state be maintained so as to be essentially free of floating debris, bottom deposits, scum, foam, or other materials, including suspended substances of a persistent nature, from other than natural sources (OAC 785:45-5-9). When numerical criteria do not apply to streams in the IRW water column conditions including nutrients are required to be maintained to prevent nuisance conditions (OAC 785:45-5-4). Aquatic life use is also a beneficial use of waters. It is called many things in different states such as fish and wildlife*

EXHIBIT R

propagation by the State of Oklahoma. Nutrient criteria in other states are being used to protect aquatic life use. Aquatic life use is measured using metrics of the biological condition of fish, invertebrate, and algae. Thus, the greater the difference in species composition of organisms between a reference and assessed site, the lower the biological condition of that assessed site and the greater the risk that aquatic life use will not be protected (sometimes called supported in regulatory jargon). The numeric and narrative criteria for protection of the fish and wildlife beneficial use in the IRW include, among others, standards for dissolved oxygen, pH, and biological criteria (OAC 785: 45-5-12). The biological criteria provides that aquatic life shall not exhibit degraded conditions as indicated by one or both of the following:

- *Comparative regional reference data from a station of reasonably similar watershed size or flow, habitat type, or fish and wildlife beneficial use subcategory designation, or*

- *Comparison with historical data from the waterbody being evaluated.*

Compliance with the biological criteria is determined based upon measures including but not limited to diversity, similarity, community structure, species tolerance, trophic structure, dominant species, indices of biotic integrity, indices of well being or other measures.

- *Metrics are measures of the biological characteristics of ecosystems that respond to effects of human activities (Karr and Chu 1999). I use the term indicators most in this report because the term metrics presumes the indicator will respond to human activities. In this study, I hypothesize that biological characteristics will respond to the direct and indirect effects of human activities, I do not presume it.*

- *Valued ecological attributes and ecosystems services are broader terms that refer to benefits gained from ecosystems (Stevenson et al. 2004, MEA 2005). These terms are only indirectly related to government regulatory processes. They do however reflect the reasons that stakeholders value ecosystems, or should value them if they were more informed. I use these as endpoints for the assessment (sensu Suter 1993) and for measurement of injury.*

When nutrient inputs are low (**Figure 1.4A**), algal growth is typically limited by the lack of nutrients. Algae provide substrate and organic carbon that feed bacteria, which are also limited when nutrient concentrations are low. Algae and bacteria interact to control the amount of DO in a stream. Algae photosynthesize, which means they use sunlight to convert water and carbon dioxide into simple carbohydrates (e.g. sugars) and DO. Both algae and bacteria respire, which means they convert sugars within their cells into water and carbon dioxide which consumes oxygen. Oxygen in streams is also supplied by diffusion from air which tends to be relatively constant when compared to changes in photosynthesis and respiration that can occur in streams. Thus, the balance in photosynthesis and respiration by microbes (algae and bacteria) regulates the DO in streams. DO is a critical resource for the breathing of invertebrates and fish, but also microbes that require the DO for respiration. When nutrient pollution is low, oxygen supplied by photosynthesis and diffusion from the air is greater than respiration's demand for oxygen, so high oxygen concentrations are maintained.

Nutrient pollution causes degradation of water quality and impairment of aquatic life and aesthetic beneficial uses by stimulating algal growth (**Figure 1.4B**). The resulting excess algal growth in streams reduces aesthetic quality for recreation (**Figures 1.1C & 1.3**). Excess algae may

EXHIBIT R

also impair recreational use of water by supporting pathogenic bacteria associated with human and animal wastes. Recent evidence from the Great Lakes regions is showing that persistence and density of microbial pathogens is greater when they are associated with algae (e.g. Ishii et al. 2006).

Aquatic life use is impaired by nutrient pollution because of several mechanisms (Figure 1.4B).

- High algal biomass can physically alter the habitat by covering the stream bottom.
- High algal biomass can have a net-negative effect on DO.
- High algal biomass can increase pH, and high pH is toxic for invertebrates and fish.
- High algal biomass and nutrients can stimulate bacteria, which respire and reduce DO.
- High algal biomass and nutrients can stimulate bacteria, which may cause disease and fouling of gills for invertebrates (Lemly 1998).

These factors will be discussed more thoroughly in the following paragraphs.

Algae can have a negative effect on DO in streams because they respire as well as photosynthesize, and these processes vary during the day (diurnally). Algae photosynthesize when exposed to sunlight. Algae respire during both daylight and nighttime periods. This diurnal variation in photosynthesis with relatively constant respiration causes variation in DO concentrations in streams. During the early daylight hours of morning, photosynthetic rates increase until they exceed respiration rates. When photosynthesis exceeds respiration, DO concentrations increase in stream water. When respiration rates exceed photosynthetic rates, DO concentrations decrease in the stream water. Thus during the day, when light intensity is sufficiently high for photosynthesis to exceed respiration rates (usually an hour or two after dawn) DO concentrations in the stream start to increase. Late in the afternoon and as the sun drops, photosynthetic rates slow, become less than respiration, and DO concentrations in the stream start to decrease. Concentrations of oxygen continue to decrease during the night until after dawn when light increases to a level that the production of DO by photosynthesis exceeds depletion of DO by respiration. So the daily DO minimum is usually around dawn and the DO maximum is late in the afternoon before dusk.

Algae can have a negative effect on DO when their abundances are high and when they stimulate bacterial accumulation. As algal abundances increase, the diurnal variation in DO concentration in streams increases. The diurnal variation in DO has been a standard method for measuring photosynthesis by algae in streams for many decades (Odum 1956). DO concentrations are commonly in the 7 to 10 mg/L range. When high algal biomasses accumulate, nighttime oxygen concentrations can decrease to less than 5, which are considered harmful to invertebrates and fish (USEPA 1986). Several other events in streams can exacerbate this low oxygen problem. Increased bacterial numbers on the algae can increase respiration significantly. Cloudy days can reduce photosynthesis and increase the duration and intensity of low oxygen periods. When seasonal changes in light and temperature cause the algae to die, oxygen demand increases greatly with a concurrent stop in oxygen supply by the dying algae. Thus, low oxygen events depend greatly on

EXHIBIT R

weather and seasonal conditions as well as nutrient pollution. Therefore, oxygen concentration may vary from day to day as well as diurnally depending upon these conditions.

The pH in streams is also affected by algae and in turn, affects species composition of all organisms in the stream. The pH is a measure of the hydrogen ion concentration (acidity) of water. It is actually the negative exponent of hydrogen ion concentration, which means that when stream acidity increases, pH as a measurement decreases. When acidity of water decreases (alkalinity increases), pH increases. The pH (acidity) in streams is buffered by a complex interaction among other chemicals in water, such as calcium carbonate. Calcium carbonate dissociates into calcium and bicarbonate and carbonate ions. These ions neutralize or buffer changes in pH. These latter ions are in equilibrium with water and carbon dioxide. The end result is that when carbon dioxide is taken up by algae during daylight times, the pH of the water can increase (see review in Wetzel 2001). Oklahoma has water quality regulations that limit the maximum pH to less than 9.0 because high pH stresses fish, invertebrates, and algae (Courtney and Clements 2004; Pan et al. 1996; Feldman and Conner 1992; USEPA 1986).

High levels of nutrient pollution almost always lead to negative effects, but low levels of nutrient pollution have complex effects. Low levels of nutrient pollution stimulate productivity of fish. Fish are larger and more abundant in streams with low levels of nutrient pollution than in more pristine streams. Low levels of nutrient pollution increase nutrient concentrations to levels that allow more species to live in the habitat. Competition among species can be reduced at nutrient concentrations that are above natural background concentrations, so more species can co-exist in the stream. In addition, some species cannot survive alone in really low nutrient conditions. Higher nutrient concentrations enable survival of these species that are not adapted to naturally low nutrient concentrations. Thus, low levels of nutrient pollution can have some positive effects on some valued ecological attributes of streams, but high levels of nutrient pollution are overwhelmingly negative. This difference in overall positive and negative effects on valued ecological attributes along environmental gradients was conceptualized by E.P. Odum in the subsidy-stress perturbation gradient (Odum, 1979).

However, negative effects on species composition (the loss of low nutrient species) are evident at low levels of nutrient pollution. Relative abundances of species in assemblages change with very slight changes in nutrient concentration. Indeed, there is evidence that some species that are adapted to low nutrients may be sensitive to environmental changes with added nutrients and they are lost from the habitat with slight increases in nutrients (Stevenson et al. in press).

As a result of the concerns for risk of losing special qualities of near natural streams, many states protect streams and lakes that are as near natural condition as is possible for a region. Wild and scenic rivers, reference-reach programs, and outstanding resource water programs are common state programs that allow special, high levels of protection for selected waterbodies. All other waterbodies are protected by lower, but satisfactory levels. The State of Oklahoma designated many segments of the IRW as a wild and scenic river in which nutrient limited conditions should be maintained (OAC 785:45-5-25(c)(7)(d)). They established a special nutrient criterion of 0.037 mg TP/L to protect this designation and aesthetics of the stream (OAC 785:45-5-19).

EXHIBIT R

1.2.3 Phosphorus

From the late 1960's to early 1970's, the role of P in regulation of algal growth in freshwater ecosystems had developed scientific acceptance and prominence. Vollenveider (1966) was calculating relationships between P loading and lake eutrophication (measured as algal biomass in the water). Stoermer and Schelske (1971) published their famous article on eutrophication, silica depletion, and predicted changes in algal quality in the journal *Science*. Legislation was passed in states around the Great Lakes to remove P from detergents to reduce phosphorus loading into the Great Lakes. Many people would argue that eutrophication was one of the key factors that stimulated the writing and passage of the Clean Water Act in 1974.

Work on nutrient limitation in lakes preceded work in streams by a few years. In his famous book *The Ecology of Running Waters*, H.B.N. Hynes (1970) wrote, "There are indications that *Cladophora glomerata* is sensitive to iron salts (Blum, 1957, 1960), and the so-called nutrient salts, potassium, nitrate, and phosphate, and for diatoms silica as well, are undoubtedly also important although we know little about their influence in running water." Borchardt (1996) charts the history of studies of nutrient limitation of benthic algae in streams. He notes that the first study was actually published in 1948, in which Huntsman describes putting a bag of fertilizer in a stream, waiting a year, returning and finding thick green algal mats growing downstream but not upstream from the bag. Starting in 1975, there was about one paper per year through 1985 showing the limiting effects of nutrients in streams. After 1985, the number of papers increased. Recent high publication rates of papers about nutrient pollution in streams show that nutrient effects in all water bodies remains an important topic as we improve our ability to quantify risk of nuisance algal growths that threaten both ecological and human health.

Much of what we know about risk of nuisance algal growths has been learned from two complementary methods, field surveys and experiments. Experiments are particularly valuable because they allow establishing cause-effect relationships. In field surveys, many environmental factors vary at the same time. For example, nitrogen (N) concentrations and silt deposition often increase with P pollution in streams, so determining whether problems in streams are due to one factor versus another requires results of experiments. Experiments have problems with simulating the real conditions in streams. Experiments are usually conducted in laboratories, over short periods of time, or in relatively small areas of a watershed. Problems with experiments occur because laboratory conditions are not exactly like streams, stream processes are highly linked upstream and downstream, and because responses of whole watersheds occur over long periods of time. So results of field surveys help us quantify relationships among valued ecological attributes and pollutants and establish pollution limits that will protect desired levels of valued ecological attributes. Experiments help us confirm the ranges of pollutants that effect valued attributes.

Many experiments have been conducted to determine if nutrients limit algal growth in streams and consequences of nutrient enrichment, but few quantify relationships so that we can determine specific effects of specific levels of nutrients. Reviews of nutrient-limitation experiments (Dodds and Welch 2000, Francoeur 2001) show that 30 to 35 percent of the streams studied were limited by nutrients. About half of those were limited by P, and the other half were limited by N. Algal growth in a few streams were limited by both low P and low N concentrations.

EXHIBIT R

Two good experimental studies for characterizing quantitative relationships between increasing P and N and algal growth are Bothwell (1985) and Rier and Stevenson (2007). Both these studies used multiple treatment concentrations in flow-through streamside channels for the experiments, which provided great fidelity to natural stream conditions and high resolution of nutrient-growth relationships. Bothwell only manipulated P concentration. His works showed great response in algal growth rates to low P concentration and then little response to increasing P concentrations above 30 µg PO_4-P/L. This asymptotic response to nutrients is predicted from nutrient uptake and algal growth experiments in the laboratory (**Figure 1.5**) and is called a Monod response, named after the scientist that proposed the relationship (Monod 1950). Rier and Stevenson (2007) observed an asymptotic response of growth rates and peak biomass of benthic algae to increasing phosphate and nitrate treatments in streamside channels. Saturating nutrient concentrations (the concentration at which higher concentrations had little stimulatory effect) were:

- 16 µg PO_4-P/L and 86 µg NO_3-N/L for growth rates (**Figure 1.6**), and
- 38 µg PO_4-P/L and 308 µg NO_3-N/L for peak biomass (**Figure 1.7**).

Saturations of growth rate and peak biomass responses were different because they were measured at different times of the colonization period. Growth rates were measured early during colonization, when there is little negative competitive effect among algae for nutrients. Peak biomass is measured later during colonization when algal abundance has reached its highest level. Peak biomass requires higher nutrient concentrations to saturate the growth rates in low biomass because nutrient supply from the water to algae decreases with higher algal biomass. Growth rates decrease and demand for nutrients increase with increasing biomass on substrates as algae accumulate after a flooding that scours algae from substrata. This is due to algal uptake rates exceeding the mixing and diffusion rates and causing a nutrient poor condition in microalgal mats. This nutrient depletion problem increases as density of algae on substrata increases (Stevenson and Glover 1993), because high densities of algae interfere with mixing of nutrient fresh waters over the algae with nutrient poor waters within pores among the microalgae on substrata. In addition, higher densities of algae take up more nutrients. Current velocity over the algae reduces this effect, because higher velocity currents increase mixing of overlying and pore waters (see review in Stevenson 1996).

Several field studies show the relationship between algal biomass in streams and total phosphorus (TP) or soluble reactive phosphorus (SRP). Dodds et al. (1997) and Stevenson et al. (2006, in press) showed algal biomass increased with increasing TP in streams of Montana, Michigan, Kentucky, and the Mid-Atlantic region of the US. Biggs (2000) showed the positive relationship between SRP and algal biomass in New Zealand streams. Lohman et al. (1992) found positive relationships between algae and nutrients in Ozark streams. Many of these relationships have been criticized because of the high variability around the predicted relationship between nutrients and algal biomass. The high variability around the relationships is not surprising given the many biological factors that affect algal biomass, such as grazing and bioturbation (disturbance by animals), and the many physical factors that affect algal biomass, such as scouring during floods, shading by tree canopies, and substratum (e.g. rock) size. Despite the high variability in biomass-nutrient relationships, these relationships are important because the magnitude of the average expected effects can be great.

EXHIBIT R

Stevenson et al. (2006) used visual observations of algae on the stream bottom to document one of the more important affects of nutrients on aquatic ecosystems, the relationship between FGA cover. Usually, algae are scraped from the stream bottom to measure algal biomass using chl a concentrations. Chl a measures do not distinguish between the different kinds of algae (diatoms, cyanobacteria, or green algae) on the stream bottom. In this case, we related percent of the stream bottom covered by *Cladophora*, a FGA, and TP, and found strong relationships between *Cladophora* percent cover and TP in both Michigan and Kentucky streams. Others have found strong correlations between algal biomass and nutrients when *Cladophora* was an important element of the algal biomass (e.g. Dodds et al. 1997), but these studies did not focus on this these algae alone. In Montana, Dodds et al. (1997) used regression and graphical analysis of a large stream database to identify acceptable levels of instream total nitrogen (TN) and TP. Review of the data in Stevenson et al. (2006) indicate a threshold in *Cladophora* cover near 0.024 mg TP/L (**Figure 1.8**). This threshold was identified by a statistical technique called CART, which will be explained later.

There has been some discussion of whether TP or SRP, an analytical estimate of PO_4, is the best indicator of P availability. Biggs (2000) usually suggests SRP and Dodds argues for TP (Dodds 2003). I recognize that both have their pros and cons. SRP is our best indicator of PO_4-P concentration in water, which is the bioavailable form. TP includes a particulate fraction of P, which is not usable until bacteria decompose the particle and release the PO_4. However, in situations where PO_4 is depleted because of high demand from algae, particulate P remains suspended in the water as a tracer and indicator that phosphate concentrations were high in the recent past. So, TP concentrations, despite their problem with including non-bioavailable P, should be more commonly correlated to recent and current PO_4 bioavailability than current PO_4 concentrations in the water over the benthic algae. Thus, the USEPA (2000) recommends use of TP in their suite of nutrient criteria measures for streams and we used TP as our primary indicator of nutrient pollution for evaluations done for this report.

In many studies (e.g. Stevenson et al. 2006), both TP and TN are related to algal biomass in streams. Based on reviews of experiments and experience in field studies, I assume that nutrients limit accrual of high algal biomasses when TP is less than 30 µg/L and TN is less than 500 µg/L. These numbers are just guidelines, but they roughly correspond to Redfield molar ratios of 16:1 (a 16N:1P molar ratio is a 7N:1P mass ratio), account for some fraction of TP and TN being in particulate form and not immediately available for uptake and growth of algae, account for potential N leakage from cells (Humphrey and Stevenson 1992), and include potential for some macroalgae having higher nutrient requirements than diatoms, which dominated most of the algal assemblages experimental systems. Thus, a key point in using N:P ratios to determine which nutrient limits algal growth is whether nutrient concentrations are low enough to limit growth.

Few published studies relate DO concentration to P concentration in large scale surveys of streams. Miltner and Rankin (1998) show a decrease in DO with increasing TP concentration in Ohio streams using data collected by the Ohio EPA. Two EPA funded projects that I supervised have shown the negative relationships between DO and TP concentrations in Michigan streams (**Figure 1.9**). In one of these studies, field crews sampled stream water and measured DO, usually between 8:00 AM and 8:00 PM. In the other study, sampling only occurred very early in the morning, no later than 3 hours after dusk, as we assumed that photosynthesis did not have enough time to increase DO concentrations. The relationship between DO and TP was significant

EXHIBIT R

and very similar in both studies, except DO was substantially lower in the early morning DO study. Both relationships were highly variable, which was expected because so many factors in streams affect DO. Both studies showed that the expected DO in a stream decreases when TP exceeds 0.040 mg TP/L.

Low dissolved oxygen caused by nutrient pollution can have negative effects on biological assemblages in streams. These can be long-term chronic effects related to reduced growth and reproduction rates and behavioral alterations (Whitmore et al. 1960, see review in Expert Witness Report of Cooke and Welch). In addition, they can be relatively short-term severe events when fish die suddenly and in large numbers. Fish kills can be caused by a variety of factors, but one is the low DO caused by nutrient pollution. These events are difficult to document and to relate quantitatively with nutrient pollution because they are relatively rare, but they are severe. When nutrients are high and water levels have gradually decreased to low flows, large biomasses of algae and associated bacteria can accumulate. In these situations, reaeration of DO is low and daily respiration can exceed photosynthesis. Therefore DO declines to low levels.

We documented one fish kill on the Illinois River that could be blamed on nutrient pollution. The ODWC blamed a fish kill on April 19, 2006 on low DO combined with spawning stress. Most fish killed were the same species, central stonerollers. An estimated 1,353 fish died in a 6,485 feet reach of stream between Round Hollow and Buck Ford (**Figure 1.10**). Our field crews went to the affected area on the day after the report of the fish kill. We also documented low DO in a diurnal study. DO was greater than 8 mg/L before 6:00 PM on April 19, but it was 0.9-2.93 mg/L between 5:30 AM and 8:00 AM on April 20. Large amounts of FGAwere observed in the river (**Figure 1.11**). Therefore, the likely cause of the fish kill was nutrient pollution causing excessive FGA, which caused the low, early-morning DO concentration.

Experiments show that production of fish and invertebrates can be limited by nutrients and algal production in streams (Dudley et al. 1986, Lamberti et al. 1989, Peterson et al. 1993, Deegan et al. 1992). Invertebrates feeding can be affected by the kinds of algae present (Fuller and Fry 1991). Abundance and composition of algal biomass affects benthic invertebrate feeding drift (Hart 1981, Kohler 1985, Kerans 1996), which increases invertebrate exposure to predation by fish. In addition, fish and herbivore grazing can reduce algal biomass in streams (Lamberti et al. 1989, McCormick and Stevenson 1989, Gelwick 2000). In some cases, predators can limit foraging of grazers and affect algal biomass (e.g. Power et al. 1985). Thus, experiments confirm that trophic interactions, from the bottom of the trophic food web to the top or from the top down, should link nutrient pollutions to potentially complex changes throughout the food web.

Three field surveys relate negative changes in species composition of invertebrates and fish to increasing concentrations of nutrients in streams. Miltner and Rankin (1998) related degradation in species composition of fish with increasing nutrients concentration using assessments of 1,657 fish-sampling sites. Effects on fish in Ohio streams were detectable when nutrients exceeded background condition in all but large rivers. They argued that P was the likely nutrient causing the problem and that problems developed when TP exceeded 0.06 mg/L. Miltner and Rankin (1998) showed little response of invertebrates from 901 sites to nutrients, except at the highest concentrations. However, all five invertebrate indicators varied significantly among groups of streams with nutrient conditions. Wang et al. (2007) show that approximately 67 percent of fish

EXHIBIT R

and invertebrate indicators tested were significantly related to N and P concentrations in 240 wadeable Wisconsin streams. Many fish indicators had threshold responses at 0.06 mg/L in Wisconsin. This threshold pattern was also observed in non-wadeable rivers of Wisconsin (Weigel and Robertson 2007). They observed thresholds in a suite of biological measures of fish at 0.06 mg TP/L and 0.64 mg TN/L.

In conclusion, strong evidence exists in studies from around the US that nutrient pollution negatively affects the ability of streams to provide full recreational benefits and support aquatic life use. Nutrient pollution releases algae and bacteria from nutrient limitation and feeds excessive growths. These excessive growths can alter habitat physically and chemically in ways that affect the species composition of invertebrates and fish in the stream. Streams covered with excessive algae and depleted of the natural balance of flora and fauna would not meet the intended management target of the State of Oklahoma for the streams of the IRW as a scenic river.

EXHIBIT R

Section 2
Water Quality and Algal Biomass

2.1 Introduction

Previous observations indicate poultry operations have increased during the last 30 years and have caused important water quality problems. A series of field studies was initiated to statistically quantify relationships among poultry house operations, stream nutrient concentrations, algal biomass, DO, pH, and species composition of algae, invertebrates, and fish. This section of the report describes the study of the relationships among poultry houses in the IRW and nutrients, algal biomass, and water quality factors that would affect species composition of algae, invertebrates, and fish. Effects of these factors on species composition of algae, invertebrates, and fish are covered in Sections 3 and 4 of this report.

Based on previous research discussed in the Background section and changes in poultry house operations in the IRW, I hypothesized that substantial nutrient pollution of IRW streams would be associated with poultry house operations. In addition, I hypothesized that nutrient pollution would stimulate growths of nuisance levels of benthic algal biomass that would alter DO and pH in IRW streams. To test these hypotheses and provide data that was specifically related to streams of the IRW, pilot and large-scale field studies were initiated during summer 2006, spring 2007, and summer 2007 which were assumed to be critical times for low-flow stressful periods (summer) and periods of peak algal biomass development (spring).

2.2 Methods

2.2.1 Sampling design

Preliminary river biological sampling was conducted during 2005 in which 13 riverine sites in Oklahoma and Arkansas were sampled. Data from these pilot studies will not be analyzed for this report because of the priority for analyzing data from three subsequent studies in which site selection and sampling methods were standardized and much greater numbers of streams were sampled to precisely quantify the analyses for this report.

Three full-scale field sampling campaigns were conducted during summer 2006, spring 2007, and summer 2007. Water chemistry and land use were determined for all sites during all three sampling campaigns. Algal biomass was determined during the summer 2006 and spring 2007 sampling campaigns. Diatom and invertebrate species composition were determined during the summer 2006 and spring 2007 campaigns, and species composition of fish was determined during the summer 2007 campaign.

During all these full-scale sampling events, sites were selected using a stratified-random sampling design in which sampling sites were selected randomly from 5 groups of potential sampling sites (the strata). Potential sampling sites were assigned to these 5 groups based on poultry house density and geographic location in the IRW. The range in poultry houses was split evenly in 5 sub-ranges or quintiles. All potential sampling sites were assigned to these bins. Then even numbers of sampling sites were selected from each quintile. Based on variability in results of previous studies

EXHIBIT R

in the literature, our goal was to sample 72 sites with the numbers of sites evenly distributed among the 5 poultry house-density groups. Because of high cost and the challenging logistics of getting sufficient numbers of trained crews, fish were sampled at 37 sites during summer of 2007. Details about the site selection process can be found in the report from Roger Olsen's Expert Witness Report (CDM 2008). **Figure 2.1** thru **Figure 2.3** are maps of sites for the three field campaigns.

During the summer 2006 and summer 2007 sampling campaigns, each site was visited and sampled one time. During the spring 2007 campaign, each site was sampled weekly for eight weeks. We sampled streams repeatedly during the spring 2007 campaign from mid-March through mid-May to characterize average conditions and the variation in conditions during a period when we expected spring rains and rapidly changing algal biomass. Data analyses were conducted with single parameter estimates, such as averages, minima, maxima, or standard deviations. The use of one estimate from each site maintained the independence in observations in the dataset, which can be a problem when repeated measures are collected from sites and used in statistical analyses.

2.2.2 Sampling, sample analysis, and map analysis

All sampling, sample analysis, and map analysis methods followed previously established methods that are commonly used by states. Details for the methods can be found in the Expert Witness Reports of Roger Olsen and Darren Brown. Below I provide an overview of these methods for the convenience of readers and to provide rationale for the parameters selected for analysis.

Habitat assessment. The sampling reach in each stream was defined as two or more riffles when sampling algae and invertebrates during summer 2006 and spring 2007. The length of the sampling reach for fish sampling was defined as 30 times the mean wetted stream width at the time of sampling, with a minimum length of 100 meters for small streams and maximum of 800 meters for large rivers. Percent canopy cover was determined with a canopy densitometer during the algal sampling events of summer 2006 and summer 2007.

Water chemistry. Water samples for nutrient analysis were collected during each site visit (summer 2006, spring 2007, and summer 2007) for analysis of TP and phosphate (PO_4) concentrations, including the eight visits during the spring 2007 campaign. Water samples for TN, nitrate-nitrite (NOx-N), and ammonia (NH3-N) were collected once from each site during each of the three field campaigns. Water samples were shipped to a laboratory for analysis. The pH, conductivity, and temperature were measured in the field with an Oakton model 300 multimeter during each site visit. DO was measured colorimetrically in the field with a Chemetrics V-2000 photometer, vacu-vials, and reagents during each site visit. Turbidity was measured with a Hach 2100p turbidometer once for all sites during each field campaign. See CDM SOP 7-5 in Darren Brown's report for details.

Algal biomass. Both planktonic (sestonic) and benthic algal samples (periphyton) were collected to determine algal biomass in the streams during the summer 2006 and spring 2007. Planktonic algal biomass was determined by filtering water through a glass fiber filter, storing it on ice, and shipping it to Aquatec laboratory for chl a analysis using a fluorometer. Algae on five 3-rock

EXHIBIT R

clusters were scrapped from known areas of each rock. Scrapings from the five 3-rock clusters were kept separate for chl a analysis to provide an estimate of the mean and variance in periphyton at sites. Sub-samples from each of the five samples collected at each site were drawn and composited into one sample for assessment of species composition from each site. The remaining proportions of each sample from the 3-rock clusters was frozen and shipped to Aquatec laboratory for chl a determination. Areas of rocks scraped and sub-sample volumes were recorded to enable calculation of chl a per unit area of stream bottom (µg chl a/cm^2) and per unit volume of stream water (µg chl a/L). See CDM SOP 7-5 in Darren Brown's report for details.

Cover of the stream bottom by FGA is an aesthetic problem. We estimated the percent cover of stream bottoms with visual observations. An area of stream was delimited using a viewing bucket or frame in which a grid of points was arranged. The grid helped quantify percent cover of the stream bottom by filamentous algae. Twenty observations were made along 5 to 10 transects in riffles of streams. The percent of filamentous algal cover and type of filamentous algae composing the cover was recorded. Subsamples of macroalgae were collected for testing and verification by a trained taxonomist in my lab at Michigan State University. Details about this protocol can be found in the Expert Witness Report of Darren Brown.

Land use and watershed area. Land use and watershed area were determined by Robert van Waasbergen for each stream site sampled. Land-use categories were determined as a percentage of basin-area based on land use classifications and maps from the 2001 National Landcover Dataset. I selected percent urban and agricultural land use in watersheds for indicators of the effects of those human activities on stream condition, which is a common approach for relating land uses to stream condition (Johnson et al. 1997; Allan et al. 1997). In addition, poultry house density (houses/mi2) was determined for each watershed. Observations of poultry waste application indicated most litter from poultry houses was put on fields close to its source (Fisher, personal communication). Thus, poultry houses outside the watershed of a stream could be contributing to P loading. That hypothesis was tested by relating P concentration in a stream to the density of poultry houses in the watershed when poultry houses within the watershed only were counted and poultry houses within 2 miles of the watershed were counted as being in the watershed. The correlation with stream P was greater when poultry houses within 2 miles of the watershed were included in the number of poultry houses in the watershed. See the Expert Witness Report of Dr. Bernard Engel for details.

2.2.3 Statistical methods

To test hypotheses systematically, I grouped variables into five categories according to their causes and their effects in the conceptual model (**Figure 2.4, Table 2.1**). These categories were: 1) land use and landscape, 2) nutrients, 3) algal biomass, 4) chemical stressors, and 5) species composition. I will address the direct and indirect effects among the first 4 categories and all causal categories in this section. For example, first I relate direct effects of land use on nutrient concentrations (section 2.3.1). In section 2.3.1, I also characterize N:P ratios to determine the most limiting nutrient. Then I study algal biomass by first relating land use to algal biomass, an indirect relationship, and then relating nutrients and algal biomass, a direct relationship (section 2.3.2). Then I study DO by first relating it to land use, an indirect relationship, and then algal biomass, a direct relationship (section 2.3.3). Finally, I study pH by first relating it to land use and then algal biomass, a direct relationship (section 2.3.4).

EXHIBIT R

The rationale for this approach follows. First, direct relationships between cause-effect factors should be the most precise and they help evaluate plausible causes, so direct relationships were evaluated. By directly causal, I mean that variation in the independent (causal) factor affects processes of the dependent (effects) factor. For example, P is directly related to algal biomass because it regulates algal reproduction, which is a process determining the state of algal biomass. Poultry house density does not directly regulate algal reproduction, thus it is indirectly related to algal biomass because it regulates processes affecting P in streams (**Figure 1.3 & Figure 2.4**). Poultry houses could be operated without adding P to streams. Indirect relationships were valuable for two reasons. One, some independent variables like land use vary less over time than nutrient concentrations, so land use could provide a more precise and accurate characterization of nutrient concentrations affecting benthic algae than actual measured nutrient concentrations in waters of the stream. Two, independent variables help us determine the causal pathway. If poultry house density in the watershed is related to nutrients, algal biomass, and DO or pH, then it is highly likely that poultry operations caused these alterations of IRW streams.

The *a priori* selected independent variables for each category were percent urban land use and poultry house density as determinants of nutrient concentrations. TP was the *a priori* variable for nutrient concentrations. FGA cover and benthic algal biomass were predicted to be determinants of DO and pH. DO and pH were expected to be the primary determinants of the species composition of algae, invertebrates, and fish. Establishing predicted relationships *a priori* help control the probabilities associated with conducting multiple statistical tests. Exploratory statistical tests were sometimes run after the *a priori* hypotheses to confirm that I did not miss anything important that would alter my conclusions.

At each successive step in the causal pathway I characterize the median, quartiles, and range of the variables, which will allow comparison with other regions. I used correlation and regression analyses to test hypotheses about relationships to determine whether they were statistically significant (unlikely to be observed by chance) and whether they are biologically significant (have a large effect). In some cases, exploratory correlations were run to ensure that key causal variables were likely the most important determinants of nutrients, biomass, or water chemistry stressors. In addition, forward stepwise regression was also run to see if combinations of variables, other than those in *a priori* hypotheses, would explain similar amounts of variation in nutrients, biomass, or water chemistry.

All variables were transformed if necessary to meet the assumptions of parametric statistical techniques. Distribution of each variable was plotted in a results file and examined to determine whether they were skewed. I used logarithmic, square root, and positive power transformations (e.g. X^2 or X^{10}) to normalize distributions of variables. Distributions of transformed variables were also plotted and transformed using another power until their distributions were not skewed. Variables with too many zeros were not included in the statistical analyses.

In addition to plotting distributions of variables before statistical tests, residuals were plotted and examined after statistical tests to ensure they were near-normally distributed. Outliers were removed from analyses in some cases. In no case were results of the statistical tests substantially different before and after outlier removal. If they were, the cause of the outlier being unusual would be recorded and discussed.

EXHIBIT R

The statistical methods used for evaluating the overall statistical significance of my analysis and individual tests are conservative. Additional injuries may be identified through the use of non-linear and multivariate statistical analysis. My opinion is based on the information that I have to date of submission of this report. I reserve the right to supplement my opinion based on review of additional data analyses and new information.

2.3 Results and Discussion

2.3.1 Nutrient concentrations

2.3.1.1 General comparisons

Finding: Nutrient concentrations were relatively high in the IRW compared to many other regions.

TP concentrations during summer 2006 ranged from 0.008 to 0.648 mg/L with a median concentration of 0.076, and 25^{th} and 75^{th} quartiles of 0.037 and 0.118 mg/L (**Figure 2.5**). SRP ranged from 0.002 to 0.590 mg PO_4-P/L with a median of 0.057 and 25^{th} and 75^{th} quartiles of 0.025 and 0.148 mg PO_4-P/L. TN ranged from 0.708 to 12.28 mg/L with a median of 2.79 mg/L and 25^{th} and 75^{th} quartiles of 1.8 and 4.23 mg/L. Nitrate-nitrite ranged from 0.10 to 9.72 mg NO_x-N/L with a median 1.27 and 25^{th} and 75^{th} quartiles of 0.37 and 2.45 mg NO_3-N/L.

TP concentrations during spring 2007 ranged from 0.007 to 1.254 mg/L, with a median concentration of 0.057, and 25^{th} and 75^{th} quartiles of 0.026 and 0.113 mg/L (**Figure 2.6**). SRP ranged from 0.001 to 1.114 mg PO_4-P/L with a median of 0.034 and 25^{th} and 75^{th} quartiles of 0.015 and 0.071 mg PO_4-P/L. TN ranged from 0.79 to 20.44 mg/L with a median of 4.05 mg/L and 25^{th} and 75^{th} quartiles of 2.42 and 6.26 mg/L. Nitrate-nitrite ranged from 0.10 to 7.58 mg NO_x-N/L with a median 1.32 and 25^{th} and 75^{th} quartiles of 0.72 and 2.77 mg NO_3-N/L.

TP concentrations during summer 2007 ranged from 0.007 to 0.945 mg/L, with a median concentration of 0.067, and 25^{th} and 75^{th} quartiles of 0.029 and 0.142 mg/L (**Figure 2.7**). SRP ranged from 0.001 to 0.925 mg PO_4-P/L with a median of 0.049 and 25^{th} and 75^{th} quartiles of 0.022 and 0.090 mg PO_4-P/L. TN ranged from 0.60 to 5.58 mg/L with a median of 2.35 mg/L and 25^{th} and 75^{th} quartiles of 1.63 and 2.88 mg/L. Nitrate-nitrite ranged from 0.10 to 5.00 mg NO_x-N/L with a median 1.64 and 25^{th} and 75^{th} quartiles of 0.81 and 2.03 mg NO_3-N/L.

The Oklahoma water quality standard for TP (0.037 mg/L) and aesthetic use was violated at 5 of 5 sites that we sampled in the Scenic Rivers sections of the Illinois and Flint Rivers. Geometric means for TP during the 8-week spring 2007 ranged from 0.069 to 0.371 mg TP/L in the Illinois and Flint Rivers. Phosphorus concentrations in Baron Fork Creek at the location during the time of sampling did not violate Oklahoma phosphorus criteria. However, Baron Fork Creek has been assessed as not meeting total phophorus criteria and aesthetic uses (OWRB 2005). Of the streams sampled during summer 2006, 73 percent were above the Oklahoma TP criterion of 0.037 mg/L. During spring 2007, 67 percent of the streams sampled had TP concentrations greater than 0.037 mg/L. During summer 2006, 62 percent of the streams sampled had TP concentrations greater than 0.037 mg/L. The streams sampled do not, however, represent a random sample of the IRW, so these percentages of streams with TP concentrations greater than the Oklahoma TP criterion are not necessarily representative of all streams. These percentages do indicate that many streams in the IRW have TP concentrations higher than the Oklahoma criterion. In a comparison with

EXHIBIT R

streams in other regions, where they were selected haphazardly or randomly, the IRW streams have higher nutrient concentrations than any of those regions.

Finding: When nutrient concentrations limited algal growth in IRW streams, P was most likely the nutrient that most limited algal growth in streams.

N:P molar ratios during spring 2006 varied from 12.9 to 1024 with a median of 77 and 25th and 75th quartiles of 37 and 142 (Figure 2.5). N:P molar ratios during spring 2007 varied from 20.9 to 1,890 with a median of 152 and 25th and 75th quartiles of 70 and 275 (Figure 2.6). N:P molar ratios during summer 2007 varied from 11.0 to 339 with a median of 77 and 25th and 75th quartiles of 41 and 143 (Figure 2.7). Leibig's law of the minimum posits that only one resource limits growth of organisms at a time. The Redfield ratio of 16:1 atoms of N versus P delineates the relative proportions of N and P that algae need to grow. When the N:P molar ratio is greater than 16, it indicates that N is in relatively greater supply than P, and P most limits algal growth. Absolute nutrient supply also affects algal growth. So if nutrient concentrations are low enough, the Redfield ratio predicts which nutrient is likely most limiting algal growth. If the nutrients concentrations are high, then nutrients do not limit algal growth regardless of the nutrient concentration. If nutrient concentrations can be depleted by organisms, for example when water levels are low and high biomasses of algae occur in the stream, then Redfield ratios predict which nutrient would be most limiting when nutrients become depleted in the future. Benchmarks for P and N limitation have been proposed at 0.030 mg TP/L and 0.50 mg TN/L. Comparing this P benchmark and the 25th percentiles of TP concentrations during spring 2007 in the IRW, algal growth in less than 25 percent of streams was probably P limited to some extent. Comparing this N benchmark and the 25th percentiles of TN concentrations, TN was not a limiting nutrient in some IRW streams.

Finding: Nutrient concentrations in IRW streams were related to poultry house density in watersheds.

Summer 2006

Regression analyses indicated that TP concentrations in IRW streams during summer 2006 were related to poultry house density, but effects were masked statistically by high TP in waters downstream from urban land use (**Figure 2.8**). TP concentrations were very weakly related to poultry house density in a multiple regression model (r^2=0.223) that included both the natural log of poultry house density and the natural log of the percent of watersheds that were urbanized:

$$\ln(TP)=-4.292+0.586*\ln(PCURB)+0.278*\ln(PHD)$$

In this model, the natural log of TP concentration (ln(TP)) was related to the natural log of the percent of urban land use in watersheds (ln(PCURB)) and the natural log of poultry house density plus 1 (ln(PHD)). One was added to poultry house density because minimum poultry house density was 0 and the natural log of 0 can not be determined. This is a standard transformation method. The coefficient associated with urban land use in the model had a small chance of being equal to zero ($p<0.001$), whereas the coefficient associated with poultry house density had a modest chance of being equal to zero ($p=0.134$). I.e. TP concentration was significantly correlated to urban land use, but not to poultry house density when using the 0.05 benchmark for attained statistical significance (p). The -4.292 intercept in this model indicates natural background

EXHIBIT R

concentrations of TP as ln(TP), i.e. when human activities are zero. Untransformed $e^{-4.292}$=0.014 mg TP/L.

If we restricted analysis to a subset of data in which urban land use was less than 10 percent of the watershed, poultry house density was significantly related to TP concentration (p=0.014) with the following equation (Figure 2.8)

$$\ln(TP)=-3.90+0.548*\ln(PHD)$$

The r^2 for this model was 0.225.

Multiple regression showed that TN was not related to poultry house density, but was significantly related to urban land use (p<0.001) in watersheds during summer 2006 (**Figure 2.9**). Even if the dataset was constrained to have less than 10 percent urban land use in the watershed to control for effects of urban influence, poultry house density was not related to TN (p=0.369, Figure 2.9). The following model had an r^2 indicating 0.29 (29 percent) of the variation in ln(TN) could be explained by ln(PCURB):

$$\ln(TN)=0.102+0.353*\ln(PCURB)$$

This model is illustrated in **Figure 2.10.**

We developed a multimetric indicator of nutrient conditions (NuteMMI) in streams for summer 2006 because of the potential for depletion of the limiting nutrient during low flow conditions that are common during summers. The NuteMMI was not related to poultry house density and was significantly related to ln(PCURB) (p<0.001) in a multiple regression model (**Figure 2.11**). However, if the dataset was constrained to watershed with low urban activity and a gradient of poultry house density, then NuteMMI was significantly related to ln(PHD) with an r^2 of 0.165 during summer 2006.

Spring 2007

Regression analyses indicated that both TP and TN concentrations in IRW streams during spring were related to poultry house density as well as the percent urbanized land use in watersheds (**Figure 2.12**).

Forward stepwise multiple linear regression with land use attributes and watershed area produced a TP model indicating the observed relationships between TP and both poultry house density (PHD) and urban land use had little probability of resulting by chance ($p\leq0.001$). The model was:

$$\ln(TP) = -4.924 + 0.581*\ln(PCURB) + 0.580*\ln(PHD)$$

The r^2 for this model was 0.339. The resulting TP model predicted that natural background TP concentration, when urban and poultry house density both equal 0, was 0.007 mg/L. It also predicted that when urban land use was at a median value, 8.2 percent, TP in IRW streams would increase from 0.025 to 0.077 mg/L when poultry house density increased from 0 to the 8.2 houses

EXHIBIT R

per mile^2 maximum. The -4.924 intercept in this model indicates natural background concentrations of TP as ln(TP). Untransformed $e^{-4.924}$=0.007 mg TP/L.

Forward stepwise multiple linear regression with land use attributes and watershed area produced a TN model indicating the observed relationships between TN and both poultry house density and urban land use had little probability of resulting by chance (p=0.019 and <0.001, respectively). The model was:

$$\ln(TN) = 0.14 + 0.367*\ln(PCURB) + 0.273*\ln(PHD)$$

In this model, the natural log of TP concentration (ln(TN)) was related to the natural log of the percent of urban land use in watersheds (ln(PCURB)) and the natural log of poultry house density plus 1.0 (ln(PHD)). The resulting TP model (with r^2=0.260) predicted that natural background TN concentration, when urban and poultry house density both equal 0, was 1.15 mg/L. It also predicted that when urban land use was at a median value, 8.2 percent, TN in IRW streams would increase from 2.47 to 4.25 mg/L with poultry house density increasing from 0 to the 8.2 houses per mile^2 maximum.

Summer 2007

Regression analyses indicated that TP concentrations in IRW streams during summer 2007 were strongly related to poultry house density (**Figure 2.13**) in a multiple regression model (r^2=0.716) that included both the natural log of poultry house density and the natural log of the percent of watersheds that were urbanized:

$$\ln(TP)=-6.118+1.083*\ln(PCURB)+1.018*\ln(PHD)$$

The coefficients associated with both urban land use and poultry house density in this model were highly significantly related to ln(TP) (p<0.001 for both coefficients).

If we restricted analysis to a subset of data in which urban land use was less than 10 percent of the watershed, poultry house density again was significantly related to TP concentration (p=0.014) with the following equation.

$$\ln(TP)=-4.269+0.994*\ln(PHD)$$

The r^2 for this model was 0.549.

Regression analyses indicated that TN concentrations in IRW streams during summer 2007 were strongly related to poultry house density (**Figure 2.14**) in a multiple regression model (r^2=0.578) that included both the natural log of poultry house density and the natural log of the percent of watersheds that were urbanized:

$$\ln(TN)=-0.717+0.417*\ln(PCURB)+0.512*\ln(PHD)$$

The coefficients associated with both urban land use and poultry house density in this model were highly significantly related to ln(TN) (p<0.001 for both coefficients).

EXHIBIT R

If we restricted analysis to a subset of data in which urban land use was less than 10 percent of the watershed, poultry house density again was significantly related to TN concentration ($p<0.001$) with the following equation.

$$\ln(TN)=-0.127+0.627*\ln(PHD)$$

The r^2 for this model was 0.569.

The NuteMMI was strongly related to poultry house density ($p<0.001$) in a multiple regression model that included ln(PCURB) (**Figure 2.15**). When the dataset was constrained to watersheds with low urban activity, then NuteMMI was again significantly related to poultry house density (ln(PHD) with an r^2 of 0.569).

Overview of nutrient relationships to land use

Our findings are consistent with the literature. Many studies relate the percent of watersheds used by humans for urban or agricultural activities to nutrient concentrations in streams (Johnson et al. 1997, Allan et al. 1997, Dauwalter et al. 2003). Recently the relationship between percent urban and agricultural land use in watersheds has been used to characterize nutrient conditions in streams (Dodds and Oakes 2004, Stevenson et al. in press). The predictions of natural background TP in IRW stream being between 0.007 and 0.014 mg TP/L is consistent with other studies. Natural background TP concentrations for streams in Michigan, Kentucky, and the mid-Atlantic region of the US were about 0.010 mg TP/L (Stevenson et al. 2006, Stevenson et al. in press).

2.3.2 Algal Biomass

Finding: Benthic algal biomass in IRW streams was higher than many other regions studied.

2.3.2.1 Range in biomass conditions

Algal biomass was measured as FGA cover of the channel bottom, chl a concentration of benthic algae, and as chl a concentration of planktonic algae. Algal concentrations were higher during spring 2007 than summer 2006. Algal concentrations were particularly high during spring 2007.

The bottom of IRW streams was covered by more filamentous algae during spring 2007 than summer 2006 (**Figure 2.16**). During summer 2006, FGA cover ranged from 0 to 85.8 percent of the bottom of streams with a median of 3.1 percent and 25th and 75th quartiles of 0 and 14.5 percent. During spring 2007, FGA cover ranged from 0 to 91 percent of the bottom of streams with a median of 20.8 percent and 25th and 75th quartiles of 10.7 and 54 percent.

The most common FGA observed in streams were in the genera *Cladophora*, *Rhizoclonium*, and *Oedogonium*. These algae were the genera that caused the most problem with extensive biomass in IRW streams. These taxa were difficult to distinguish from each other because they feel rough in the field. So I grouped into a taxonomic category, FGA for use in this study. Green algae actually refers to a taxonomic phylum of algae called Chlorophyta, which includes other FGA that were observed in the IRW streams, such as genera *Spirogyra*, *Mougeotia*, and *Zygnema*, which are in the order Zygnematales. *Draparnaldia*, another FGA, was also observed in IRW streams. The latter four genera could be distinguished from this nuisance bloom forming group, the FGA genera *Cladophora*, *Rhizoclonium*, and *Oedogonium*. *Spirogyra*, *Mourgeotia*, *Zygnema*, and *Draparnaldia* are

EXHIBIT R

slippery-feeling, so they are easy to distinguish from the rough-feeling *Cladophora, Rhizoclonium, and Oedogonium*. *Spirogyra, Mougeotia, Zygnema*, and *Draparnaldia* have considerable slippery mucilage on their cell walls, plus they tend to occur in low nutrient waters and seldom cause extensive cross-stream nuisance blooms as a result of nutrient enrichment. *Spirogyra, Mougeotia, Zygnema*, and *Draparnaldia* were not included in the FGA calculation because they do not form blooms in IRW streams and could be distinguished from the bloom-forming FGA (*Cladophora, Rhizoclonium*, and *Oedogonium*). Thus, in this report, I only refer to (*Cladophora, Rhizoclonium*, and *Oedogonium*) when I use the phrase, "filamentous green algae" and the acronym FGA. *Vaucheria* and *Batrachospermum* are also filamentous algae, but they are not in the phylum Chlorophyta. *Vaucheria* and *Batrachospermum* were observed in IRW streams, but not in blooms. *Vaucheria* and *Batrachospermum* are distinguished easily from *Cladophora, Rhizoclonium, and Oedogonium*. Test of field crew's field identifications indicated they accurately distinguished the FGA (*Cladophora, Rhizoclonium*, and *Oedogonium*) groups from other algae.

Benthic algal biomass was greater during spring 2007 than summer 2006 (Figure 2.16). During summer 2006, benthic algal biomass ranged from 0.007 to 13.8 µg chlorophyll *a*/cm² with a median of 3.79 and 25th and 75th quartiles of 2.5 and 6.2 µg/cm². During spring 2007, benthic algal biomass ranged from 0.2 to 33.5 µg chlorophyll *a*/cm² with a median of 4.9 and 25th and 75th quartiles of 1.68 and 7.85 µg/cm².

Planktonic algal biomass was approximately equal during spring 2007 and summer 2006 (Figure 2.16). During summer 2006, planktonic algal biomass ranged from 0.2 to 15 µg chlorophyll *a*/L with a median of 1.2 and 25th and 75th quartiles of 0.65 and 2.15 µg/L. During spring 2007, planktonic algal biomass ranged from 0.1 to 20 µg chlorophyll *a*/L with a median of 1.45 and 25th and 75th quartiles of 0.8 and 2.35 µg/L. Based on this range of conditions, waters were usually relatively clear with less than 1.45 µg chlorophyll *a*/L, but would sometimes be murky with chlorophyll as high as 20 µg/L.

2.3.2.2 Discussion

We can estimate natural FGA cover using a couple methods. **Figure 2.21** shows that FGA cover is usually less than 10 percent when TP concentration is less than 0.020 mg/L. We can also set urban land use and poultry house density to zero and predict natural FGA cover, which is predicted to be close to 0.2 percent. From practical experience, many natural streams have no FGA cover, but some can occur in small patches where nutrient rich groundwater is leaking into the surface water of the stream (Valett et al. 1994).

The percent cover of IRW streams with FGA was higher than in Kentucky and Michigan streams (Stevenson et al. 2006). A seasonal average of fifty percent coverage of stream bottoms is considered very high. About 25 percent of the sampled IRW streams during spring 2007 had greater than 50 percent of the stream bottom covered by FGA.

Welch et al. (1988) synthesized results of literature and considered nuisance algal biomass to be greater than 20 percent FGA cover or 100 to 150 mg chlorophyll *a*/cm². Surveys of Montana voters and recreational users of the Clark Fork River were used to determine an acceptable biomass of algae in rivers to support human uses (Tepley draft, http://www.umt.edu/watershedclinic/algaesurveypix.htm). They found dramatic decreases in

EXHIBIT R

desirability above a 150 mg chl a/m^2 (=15 µg chl a/cm^2) benchmark. Although qualitative relationships between aesthetics and conditions threatening biodiversity have been known, quantitative relationships have been lacking. Figures **2.17** to **2.20** show IRW streams with approximately, 10, 20, 50 and 90 percent cover of the stream bottom by FGA.

Our results show that 50 percent of our sampled streams had greater than 20 percent FGA cover. Our results indicate that 20 and 10 percent of streams sampled during spring 2007 had greater than 100 and 150 mg chlorophyll a/cm^2, respectively (Figure 2.21).

I estimated the proportion of the stream miles in the watershed that would be predicted to have FGA algal cover greater than 20 percent during the spring period when FGA grows well in streams. Usually, this is a 3 to 4 month period when water temperature and flow are satisfactory for growth of *Cladophora, Rhizoclonium*, and *Oedogonium*. We have an assessment of the land use and poultry house densities in all 3rd order watersheds throughout the watershed. Using that assessment and the relationship between ln(PHD) and ln(PCURB) above, I calculated the average FGA cover expected in the 332 watersheds. The maximum predicted values were near 57 percent and the minimum was close to 0 percent. Thirty-one percent of the 3rd order streams in the watershed were predicted to have greater than 20 percent average FGA cover. This is a reasonable estimate to compare to the 50 percent of the stream sampled in the watershed. The sampled streams were randomly selected from quintiles of poultry house density, so they represent the range of conditions, but do not provide a statistical sampling of streams in the watershed. On average, this indicates that 3rd streams in the watershed were in better shape than our sample set. In other words, there were more watersheds with low poultry house densities than high densities. The 31 percent estimate of stream miles in the watershed with greater than 20 percent FGA cover probably holds for larger and smaller streams as well. Interactions with temperature, flow, and nutrient loading probably counter balance effects of stream size for this range of FGA cover. I've seen smaller streams as well as larger rivers covered with FGA.

Finding: Algal biomass in IRW streams was related to TP concentrations and poultry house density as well as urban land use.

All algae - Summer 2006

Cover of the stream bottom by FGA was not related to poultry house density or urban land use during summer 2006 ($p>0.190$, **Figure 2.22**). Benthic algal biomass was related to urban land use ($p=0.013$) but not to poultry house density during summer 2006 ($p=0.429$, **Figure 2.23**). Planktonic algal biomass was not related to urban land use ($p=0.263$), but was related to poultry house density ($p=0.053$, Figure 2.19).

Both planktonic and benthic algal biomass were significantly related to TP concentrations ($p<0.05$, **Figure 2.24**). Biomass of FGA was not related to TP concentrations in streams ($p=0.313$).

The differing responses of benthic and planktonic algae to land use may be related to urban wastewater discharge. Planktonic algae tend to develop in streams during low flow conditions when water stays in the stream for long periods and the planktonic algae have time to accumulate. When flows are high, they are washed out of the stream before they can accumulate. Benthic algae are just the opposite; flow stimulates growth of benthic algae because currents replenish nutrients

EXHIBIT R

within the algal mats (Stevenson and Glover 1993, Stevenson 1996). Urban wastewater discharge is often an important source of flow in streams during summers when stream flows are naturally low. Urban wastewater discharge supplements flows in these streams. Thus, urban wastewater discharge with associated nutrients may stimulate benthic algae in watersheds with such discharges, but plankton develop in streams in which urban wastewater discharge is low and poultry house nutrient enrichment is high. Development of planktonic algae in streams can decrease water transparency and aesthetics of the streams.

Filamentous green algae - Spring 2007

FGA cover was significantly related to poultry house density (PHD) ($p<0.001$) and percent urban land use ($p<0.001$) (**Figure 2.25**).

$$\mathrm{sqr(FGA)}=0.288+1.515*\ln(\mathrm{PHD})+1.220*\ln(\mathrm{PCURB})$$

The square root of FGA cover is represented by sqr(FGA). The amount of variation in the square root of FGA cover explained by this model is 24.91 percent (i.e. $r^2=0.249$). Although the percent of agricultural land use was positively related to FGA cover, it was not after effects of poultry house density were accounted for. Watershed area was not significantly related to FGA cover.

FGA cover was more highly correlated with P concentrations than N (**Table 2.2**). FGA cover also was more highly correlated with TP than with soluble or dissolved fractions of phosphorus (SRP, TDP). Both Cladophorales and FGA cover were similarly and significantly correlated to P concentrations ($p<0.05$) (Table 2.2, Figure 2.25). The square root of FGA cover (sqr(FGA)) was significantly related to TP concentration alone ($p<0.001$) in the following model:

$$\mathrm{sqr(FGA)}=9.034+1.430*\ln(\mathrm{TP})$$

The amount of variation in FGA cover explained by this model is 28.7 percent (i.e. $r^2=0.287$). The pattern in the FGA along the TP gradient in Figure 2.25 indicated a threshold in FGA cover. Results from this analysis will be presented below.

Benthic algal biomass as chlorophyll a/cm²

Benthic algal biomass (chlacm2, µg chl a/cm^2) was significantly related to poultry house density (PHD, houses per mile^2, $p=0.001$) and percent urban land use ($p<0.001$) (**Figure 2.26**) in an analysis using forward stepwise multiple linear regression.

$$\ln(\mathrm{chlacm2})=-1.024+0.560*\ln(\mathrm{PHD})+0.706*\ln(\mathrm{PCURB})$$

39.1 percent of the variation in ln(chlacm2) was explained by this model (i.e. $r_{adj}^2=0.391$). Agricultural land use was also related to benthic algal biomass, but not after variation associated with poultry house density was accounted for. Watershed area was also not related to benthic algal biomass.

Benthic algal biomass was significantly related to TP concentration ($p<0.001$) in the resulting model based on both simple linear regression and forward stepwise MLR. The following model explained 37.7 percent (i.e. $r_{adj}^2=0.377$) of the variation in benthic algal biomass:

EXHIBIT R

$$\ln(chlacm2)=3.109+0.640*\ln(TP)$$

Benthic algal biomass was more highly correlated with P concentrations than nitrogen (**Table 2.3**, Fig. 2.22). Benthic algal biomass was also more highly correlated with TP than with SRP or TDP.

Planktonic algal biomass

Variation in planktonic algal biomass (chlal, µg chl *a*/L) was related to poultry house density, both alone and in combination with percent urban land use and watershed area (**Figure 2.27**). In a regression model with only poultry house density, 20.3 percent of the variation in plankton biomass was explained. However the model resulting from analysis with forward stepwise MLR, poultry house density, percent urban land use, and watershed area (areami2) were statistically significant ($p=0.001$, $p<0.001$, and $p<0.001$, respectively). This latter model explained 43.0 percent of the variation in ln(chlal):

$$\ln(chlal)=-1.968+0.461*\ln(PHD)+0.385*\ln(PCURB)+0.263*\ln(areami2)$$

In the analysis of relationships with direct regulatory factors using forward stepwise MLR, planktonic, algal biomass was related positively to TP and watershed area and negatively to total dissolved P ($p<0.001$ for all coefficients associated with all independent variables):

$$\ln(chlal)=0.844+1.999*\ln(TP)-1.389*\ln(TDP)+0.240*\ln(areami2)$$

This model explained 51.1 percent of the variation in planktonic algal biomass as ln(chl a/L) (i.e. $r^2=0.511$). Both SRP and TDP were positively related to planktonic algal biomass if TP was not in the model, but if TP was added to the model, both were negatively related to algal biomass. This provides evidence for soluble nutrients being taken up by algae. TDP was selected for the model above because it was correlated slightly better than SRP to planktonic algal biomass in the forward stepwise selection of variables.

When independent variables were constrained to TP and watershed area (**Figure 2.27**), both were significantly related to planktonic algal biomass ($p\leq0.001$) in the following model:

$$\ln(chlal)=0.904+0.451*\ln(TP)+0.231*\ln(areami2)$$

This model explained 40.1 percent of the variation in planktonic algal biomass as ln(chl a/L) (i.e. $r_{adj}^2=0.401$), which is less than the model that includes TDP, but TDP was not modeled for analysis of injury.

Watershed area affects planktonic algae differently than benthic algae (Vannote et al. 1983). Planktonic algal biomass should increase with watershed area because residence time of water in the watershed increases with size of the watershed upstream from the sampling point (Stevenson in press). With longer residence time, more algae have time to grow and accumulate. Thus, larger rivers have more plankton in them naturally than small streams, but natural levels in both rivers and streams are not nuisance levels of algae.

Discussion

EXHIBIT R

The stronger relationship between benthic algal biomass and nutrient concentrations in the spring than summer that we found was expected because that is the season of year when FGA grow in streams. During spring, there is less nutrient depletion than summer masking the relationship between algae and nutrients in the higher flows of spring than summer. In addition, water temperature and water flow during spring is more conducive for FGA growth during spring than summer.

2.3.2.3 Threshold effects on algal biomass

Finding: A threshold in response of FGA to increasing P concentrations occurred at 0.027 mg TP/L during spring 2007.

Thresholds were evaluated in algal biomass responses along the TP gradient using CART analysis. A CART changepoint in FGA cover was identified at 0.027 mg TP/L, with a difference in average FGA cover of 4 percent in TP concentrations less than the change point and 36 percent FGA cover above the change point (Figure 2.25). The CART relationship explained 42.7 percent of the variance in FGA cover (Figure 2.25). This compared to 28.7 percent of explained variance in FGA cover by a linear regression model with TP concentration.

A changepoint in benthic algal biomass was identified at 0.029 mg TP/L, with a difference in average biomass of 1.3 and 5.4 µg chl a/cm^2 below and above the changepoint (Figure 2.26). The CART relationship explained 32.8 percent of the variance in benthic algal biomass. This compared to 39.1 percent of explained variance in benthic algal biomass by a linear regression model with TP concentration.

A changepoint in planktonic algal biomass was identified at 0.047 mg TP/L, with a difference in average biomass of 0.6 and 2.3 µg chl a/L below and above the changepoint (Figure 2.27). The CART relationship explained 41.3 percent of the variance in plankton biomass. This compared to 31.2 percent of explained variance in planktonic algal biomass by a linear regression model with TP concentration.

The amount of variation explained by CART analysis was considerably greater than by linear regression for FGA cover and planktonic algal biomass, but not for benthic algal biomass. In addition, graphic patterns show changepoints more distinctly for FGA cover and planktonic algal biomass than for benthic algal biomass. Therefore, there is greater risk that sudden, punctuated responses to increasing TP concentration will be observed for FGA cover and planktonic algal biomass than for benthic algal biomass. Thus punctuated responses, thresholds, in FGA cover and planktonic algal biomass were observed at 0.027 and 0.047 mg TP/L, respectively.

2.3.3 Turbidity

Finding: Water column turbidity was related to poultry house density as well as urban land use, but values were not high during the sampling period and in the smaller streams that were sampled.

Relationships between turbidity (measured in nephalometric turbidity units) and land use and nutrients were evaluated with results from summer 2006, because turbidity associated with planktonic algae and nutrient pollution is most likely to develop in low flow conditions that are

EXHIBIT R

common during summers. Turbidity was related to both poultry house density and urban land use with the following model (**Figure 2.28**):

$$\ln(\text{turbidity})=-0.095+0.465*\ln(\text{PHD})+0.305*\ln(\text{PCURB})$$

The r^2 for this model was 0.097, which is relatively low indicating a lot of variability in the data could not be explained by the model.

To estimate the magnitude of these effects over the range of poultry house densities in the IRW, we calculated the expected turbidity with percent urban land use set to the median value and poultry house density set to zero and the maximum value. Turbidity is predicted to change from 2.0 to 5.3 NTUs over the range of 0-8 poultry houses/mi^2 and when urban land use was the median value, 14 percent. Turbidity of 5 NTUs is considered visible, but only above 10 is usually considered elevated.

We suspected that watershed area would be an important predictor of turbidity, because phytoplankton densities usually decrease downstream as they have more time to accumulate. However, neither turbidity nor phytoplankton density during the summer 2006 sampling increased with stream size (watershed area). In fact, turbidity measured as NTUs was not correlated phytoplankton concentrations.

2.3.4 Dissolved oxygen

2.3.4.1 DO general patterns

Finding: DO concentrations in streams were commonly less than Oklahoma standards supporting aquatic life use during the summer.

Average DO in streams during the summer 2006 sampling period was 5.2 mg/L with a minimum of 0.9, 25th and 75th quartiles of 4.3 and 6.2, and a maximum of 9.2. Thirty of sixty-nine streams had DO concentrations less than 5.0 mg/L, which is the DO criterion for warm water aquatic communities during summer conditions (**Figure 2.29**). Even more streams violate the DO criteria for cool water aquatic life use which is 6.0 mg/L during summer conditions (OAC 785: 45-5-12).

DO concentrations in streams over the 8-week spring 2007 sampling period were higher than during summer, with an average of 10.1 mg/L, a minimum of 7.6, 25th and 75th quartiles of 9.3 and 10.8, and a maximum of 13.4 (Figure 2.29).

The cumulative frequency distribution for DO during summer 2007 showed few streams with DO less than the 5.0 mg/L benchmark (**Figure 2.30**). The higher DO range during summer 2007 than summer 2006 is likely due to the mid-day sampling of DO by the fish crews during summer 2007 versus the early morning DO sampling by the water chemistry crews during summer 2006.

2.3.4.2 DO - Summer 2006

Finding: DO concentrations in streams were related to poultry house density in watersheds during summer 2006.

EXHIBIT R

DO during summer 2006 was complexly related to land use (**Figure 2.31**). In a two factor multiple regression analysis, DO was positively related to urban land use and not significantly related to poultry house density. The positive relationship between DO and urban land use is likely due to the increased flow from wastewater discharge and stimulation of benthic algae that was noted above.

The statistical masking of poultry house effects on DO by urban effects became evident, if the dataset was limited to streams with less than 10 percent urban land use in watersheds. DO was negatively related ($p=0.075$) to poultry house density with the following model if the dataset was limited to streams with less than 10 percent urban land use in watersheds:

$$DO(mg/L)=5.57-0.92*\ln(PHD+1)$$

This model predicts that DO in streams would be 5.57 mg/L when PHD was 0 and it would 3.7 mg/L when PHD was 8.2, the maximum PHD studied (Figure 2.31). Thus, the predicted DO in streams with PHD at maximum concentrations is less than the Oklahoma DO criterion for warm water aquatic communities.

By setting expected DO to 5.0 mg/L, we can predict the impact level of PHD that would cause violation of Oklahoma water quality standards. If we represent the standard linear model with the following expression:

$$Y=a+bX$$

We can rearrange the equation and solve for X:

$$X=(Y-a)/b$$

Since we have a special case where X really is PHD+1 and is natural log transformed in the model, we need to account for this with the following:

$$\ln(X+1)=(Y-a)/b$$

Then :

$$X=e^{(Y-a)/b}-1$$

According to the last relationship, X would equal 0. 0.85812 poultry houses per square mile, if Y=5.0 mg/L, a=5.57 mg/L, and b=-0.92. Thus, if the landscape contamination is greater than 0.85812, then we would expect stream DO to be less than 5.0 mg/L.

DO in streams during summer 2006 was not related to TP concentration when all streams were included in the analysis. However, when I limited the streams in the data set to streams with low percentage of urban land use in the watershed, DO was significantly negatively related to TP concentration (**Figure 2.32**). The negative relationship between nutrients and DO in large scale survey was also noted in unpublished research from Michigan (Stevenson unpublished data, Figure 1.9). This relationship was also observed in reference streams in Ohio by Miltner and Rankin (1998).

EXHIBIT R

2.3.4.3 DO - Spring 2007

Finding: Variability in DO increased with increased algal biomass and TP during spring 2007.

The standard deviation in stream DO during the 8-week spring 2007 sampling period averaged 1.8 mg/L, with a minimum of 0.4, 25th and 75th quartiles of 1.2 and 2.3, and a maximum of 3.7 mg/L. The minimum DO observed in each stream over the eight week period averaged 7.6 with the lowest value being 4.1, 25th and 75th quartiles of 6.9 and 8.5, and the highest minimum being 9.7 mg/L. DO concentrations less than 5, 4, and 3 mg/L become increasing stressful to fauna. Therefore, absolute concentration of DO was seldom stressful to aquatic life in IRW streams during spring. DO concentrations are usually measured for assessment of criteria during the low flow conditions of summer when concentrations are usually lowest. However, the high diurnal variability in DO for some sites indicated by the standard deviation in DO among sampling dates may have been stressful for fish and aquatic invertebrates.

DO concentration varied with time of day that sites were sampled. Sites sampled early and late during the day had lower DO than during mid-day (**Figure 2.33**). The diurnal pattern in DO is due to the diurnal variability in photosynthetic rates of algae in streams and the relatively constant rate of respiration by algae, bacteria, and animals in streams.

The standard deviation in DO of streams during the eight weeks of sampling was positively related by multiple linear regression to the amount of algae in streams (**Figure 2.34**), thus the standard deviation in DO increased significantly ($p<0.001$) with benthic algal biomass and FGA biomass, independently. The standard deviation in DO in a stream is an indicator of diurnal variability in DO because streams were sampled in different orders on different sampling days. The standard deviation in DO also increased significantly ($p<0.001$) with TP concentrations as well as poultry house density and percent urban land use in watersheds upstream from the sites sampled (**Figure 2.35**). This is certainly the consequence of the stimulation of algal growth and biomass by P, resulting in increased photosynthetic rates with increasing algal biomass, and increased diurnal variability in DO in streams.

Average DO measured in a stream was also positively related to benthic algal biomass ($p=0.003$), FGA cover ($p<0.001$), TP concentration ($p=0.05$), and poultry house density and percent urban land use in watersheds ($p\leq0.002$) (**Figure 2.36**). Minimum DO concentrations were not clearly linked to higher algal biomass or poultry house density, but they were related negatively ($p=0.001$) to TP concentrations in streams (**Figure 2.37**). We expect minimum DO to decrease with increasing algae because respiration by live algae and the bacteria associated with living and dead algae should increase. The lack of major change in minimum DO indicates that respiration and photosynthesis was relatively balanced.

2.3.5 pH

Finding: Average and maximum pH in streams were high and increased with poultry house density and effects.

The pH is a measure of the negative exponent of the hydrogen ion concentration in streams. So low pH is acidic water and high pH is alkaline water. Loading of acid and alkaline substances can affect stream pH. Stream pH can also be affected by carbon dioxide uptake by algae in streams

EXHIBIT R

during the day. This causes alkalization of the water as carbon dioxide is consumed and hydrogen ion concentration decreases. Thus, high algal biomasses may cause alkalization of stream waters. We did not, however, observe significant variation in pH with time of day, as we did DO (Figure 2.33).

Average pH in IRW streams during the 8-week spring 2007 sampling period was 7.8, with a 6.9 minimum, 25th and 75th quartiles of 7.6 and 8.0, and a maximum of 9.1 (**Figure 2.38**). The maximum pH in an IRW stream during the spring 2007 had an average value of 8.8 with the lowest maximum of 7.2, 25th and 75th quartiles of 8.2 and 9.35, and the highest pH maximum of 10.6. These maximum values of pH are higher than 9.0 more than 25 percent of the time (**Figure 2.39**).

The pH values of 8.5 and 9.0 are water quality criteria for Georgia, Tennessee, Iowa, and Oklahoma. Oklahoma's rules state that, "The pH values shall be between 6.5 and 9.0 in waters designated for fish and wildlife propagation; unless pH values outside that range are due to natural conditions (OAC 785:45-5-12(f)(3))."

Twenty-six of sixty-five streams with 7 or measurements of pH during the spring 2007 sampling period had 1 or more pH observations greater than 9.0 (Figure 2.39), i.e. maximum pH at 26 of 65 streams was greater than 9.0 during spring 2007. Eight of that set of sixty-five streams had 2 or more pH observations greater than 9.0.

The high pH in IRW streams was not due to natural causes. Average pH at sites increased with increasing benthic algal biomass ($p=0.118$), FGA cover ($p=0.001$), TP concentration ($p=0.013$), urban land use ($p=0.03$), and probably poultry house density ($p=0.166$) (**Figure 2.40**). Maximum pH observed at sites was better correlated with these causal variables than average pH. Maximum pH at sites increased with increasing benthic algal biomass ($p<0.001$), FGA cover ($p=0.005$), TP concentration ($p=0.004$), and urban land use ($P<0.001$), and poultry house density ($p=0.037$) (**Figure 2.41**).

EXHIBIT R

Section 3
Biodiversity of Benthic Diatom and Macroinvertebrates

3.1 Introduction

Benthic diatoms and macroinvertebrates are important elements of stream food webs (Hynes 1970, Allan and Castillo 2007). They represent valued elements of biodiversity by the public. Many public watershed groups use benthic macroinvertebrates in their sampling programs. As the public learns more about diatoms, the value of this group has increased because of their beauty, ecological importance, and biodiversity.

Benthic diatoms and macroinvertebrates are used by many states of the US to assess the biological condition of streams and determine aquatic life use support. Biological assessment of state waters is mandated by the USEPA because of two goals of the Clean Water Act: protecting fish, shellfish, and wildlife; and restoring biological integrity. Almost all states and tribes use benthic macroinvertebrates in their sampling programs. A growing number of states and tribes use diatoms as well as other algae in their environmental monitoring program. This number is growing because of the sensitivity of diatoms to nutrients, a nationally recognized problem, and the developing number of scientists that can provide the technical expertise for diatom programs.

I used the species composition of benthic diatoms and macroinvertebrates to calculate indicators of biological condition to measure injury. I hypothesized:

- The number of reference taxa and individuals (those that occur at the least disturbed reference) that are sensitive to pollution (both benthic diatoms and macroinvertebrates) will decrease with increasing poultry house density in watersheds, nutrient concentrations, and nutrient-related stressors in streams;
- The number of taxa and individuals that are tolerant to pollution will increase with increasing poultry house density in watersheds, nutrient concentrations, and nutrient-related stressors in streams.
- The trophic structure of invertebrate assemblages will change with increasing poultry house density in watersheds, nutrient concentrations, and nutrient-related stressors in streams.

3.2 Methods

3.2.1 Sample site selection

Benthic diatoms and macroinvertebrates were sampled during the summer 2006 and spring 2007 sampling campaigns. Sampling sites were selected using a stratified random approach which insured that sampled sites represented the full gradient of poultry house activities. Approximately 70 sites were sampled during each field campaign. Details of site selection are above in section 2.2.1 and in the Expert Witness Report of Roger Olsen.

EXHIBIT R

3.2.2 Sampling and sample analysis

Benthic diatoms were sampled by scraping five 3-rock clusters at each site. A subsample from each cluster was placed into one container and preserved with formalin for transport to the lab for identification. Macroinvertebrates were sampled from the richest targeted habitat, usually the riffle, at three randomly selected locations in the reach. Macroinvertbrates were sampled by agitating a 1 meter square area upstream from a kick net using the stream current to transport the invertebrates downstream into the net. Macroinvertebrates in the net were transferred into a quart jar, preserved in isopropyl alcohol, and shipped to a lab for identification.

Benthic diatoms were identified by staff in my laboratory at Michigan State University. My laboratory has processed thousands of samples for national and state programs, as well as my own research. I have an extensive library of books and papers on diatom taxonomy and also the high quality microscopes and camera systems needed for identifying, counting, and documenting the diatoms studied. We used standard methods of acid cleaning samples, mounting them in NAPHRAX, and then identifying 600 valves of diatom to the lowest taxonomic level. Valves are parts of diatom cell walls (Figure 1.1A). The glass cell walls of diatoms have two valves that fit together like Petri dishes. The shapes and markings of the cell walls are used to identify diatom species. Almost all valves were identified to species or variety levels.

All macroinvertebrates in samples were identified to the lowest possible taxonomic level by staff in the laboratories of Dr. Richard Merritt at Michigan State University and Chadwick and Associates. Identification of macroinvertebrates to species level is difficult for many groups because larvae often do not have all the features needed for identification to that level. As a result, species composition of samples was often a mixture of taxa identified to order, family, genus, and species level.

3.2.3 Data analysis

We used the relative abundances of taxa (order, family, genus, or species) in samples and the pollution tolerance and sensitivity of those taxa to calculate indicators of species composition that reflect the biological condition of assemblages in the streams. The pollution tolerance and sensitivity characterizations for taxa were determined from literature sources (**Table 3.1**).

These indicators were calculated using the following formula:

$$\Sigma\, p_i \Theta_i / \Sigma p_i$$

Where p_i is the proportion (ranging from 0 to 1) of individuals in a taxon for which pollution sensitivity and tolerance is known, Θ_i is the pollution sensitivity or tolerance of a taxon. Thus, this calculation is not biased due to lack of ecological knowledge about some taxa in samples.

Trophic structure of invertebrate assemblages was assessed with the number of taxa and proportion of individuals in different functional feeding groups. Functional feeding groups indicate the type of food that invertebrates eat and how they eat it. Predators eat other animals. Filter collectors and gathering collectors tend to feed from the water column and substratum respectively by collecting suspended or loosely attached settled organic matter. Scrapers (or grazers) scrape benthic algae from substrata. Shredders can eat large pieces of organic material

EXHIBIT R

like leaves. They do this by shredding the material, leaving fine particles of organic material that are eaten by collectors.

The indicators of species composition (**Table 3.1**) were then related to land use and stressors related to nutrient pollution to link human activities, stressors, and ecological injury. Indicators were transformed with natural log, square root, or positive power transformations to normalize the data for parametric statistical analyses. Correlation and regression analyses were used to quantify these relationships and test the hypotheses:

- The number of taxa and individuals sensitive to pollution, both benthic diatoms and macroinvertebrates, will decrease with increasing poultry house density in watersheds, nutrient concentrations, and nutrient-related stressors in streams;
- The number of taxa and individuals that are tolerant to pollution will increase with increasing poultry house density in watersheds, nutrient concentrations, and nutrient-related stressors in streams.
- The trophic structure of invertebrate assemblages will change with increasing poultry house density in watersheds, nutrient concentrations, and nutrient-related stressors in streams.

The algal and invertebrate indicators of species composition and trophic structure were related to urban land use and poultry house density using a multiple regression model with the later variables as independent variables. These biological indicators were related to stressors, such as TP, DO, pH, and FGA cover (habitat alteration) using correlation analyses. For invertebrates, these relationships between land use, stressors with species composition indicators were determined for summer 2006 and spring 2007 separately, and then for all sites during each of these times and only those sites with low levels of urban activities in watersheds. A low level of urban activities in watersheds was defined as less than 10 percent urban land use and little exposure to effluent from waste-water treatment plants. Urban land use was not included in the regression models relating invertebrate indicators to poultry house density when using the dataset in which urban land use was restricted to less than 10 percent.

Multiple hypothesis tests increase the risk of finding significant results because of the repeated testing. The relative number of significant tests compared to all tests will be used to evaluate the overall statistical significance of my analysis of species composition change as a result of increasing poultry house density in watersheds, nutrient concentrations, and nutrient-related stressors in streams.

The statistical methods used for evaluating the overall statistical significance of my analysis and individual tests are conservative. Additional injuries may be identified through the use of non-linear and multivariate statistical analysis. My opinion is based on the information that I have to date of submission of this report. I reserve the right to supplement my opinion based on review of additional data analyses and new information.

EXHIBIT R

3.3 Results and Discussion

3.3.1 Benthic algae

3.3.1.1 Results

Finding: Species composition of benthic diatoms was affected by P during summer 2006 and spring 2007.

Species composition of diatom assemblages was strongly affected by TP concentration in IRW streams. Six of nine metrics during summer 2006 and all 9 metrics during spring 2007 were correlated with TP concentrations in streams ($p<0.05$, Bonferroni corrected one-tailed test, Table 3.1). All metrics responded in ways that they were expected to respond to P.

The MAIATSI increased from about 2 to greater than 5 during both summer 2006 and spring 2007, with somewhat lower MAIATSI values in low P conditions during spring than summer (**Figure 3.1**). The percent low P individuals ranged from 80 to 100 percent in low P streams to between 0 and 20 percent in high P streams. The percent high P ranged from about 10 to 20 percent in low P to 70 to 80 percent in high P. Many species are classified as intermediate P species and not included in these calculations. These ranges in response of low and high P diatoms to the P gradient in IRW streams is greater than the response to the range of conditions in the hundreds of streams studied in the mid-Atlantic Highlands (Stevenson et al. in press).

Finding: Species composition of benthic diatoms was related statistically to poultry house densities during spring of 2007, but not as greatly during summer 2006.

Only two metrics were significantly related to poultry house density in simple correlations (Table 3.2) (**Figure 3.2**), unless we accounted for the percentage of urban land use in watersheds (**Table 3.3**). When diatom metrics were related to poultry house density with a multiple linear regression models that also included the percentage of urban land use, three metrics during summer 2006 and all metrics during summer 2007 were significantly related to poultry house density ($p\leq0.032$).

3.3.1.2 Discussion

The results showed that as TP in streams and poultry house density in watersheds increased, there was a decrease in the probability of observing individuals in species that were capable of living in low nutrient concentrations. There was an increase in the percentages of individuals in species that required high nutrient conditions to survive. Thus elevated P concentrations associated with poultry house operations enable invasion of microbes that did not occur in large numbers in those habitats naturally, which probably reduced the survival of native individuals adapted to low nutrients.

The lack of correlation of diatom indicators to ln(TP) during summer 2006 indicates measured TP did not reflect the nutrient exposure of streams.

3.3.2 Benthic macroinvertebrates

3.3.2.1 Benthic invertebrate indicators of species composition

Finding: Species composition of benthic macroinvertebrates was affected by factors associated with algal biomass accumulation and poultry house wastes.

EXHIBIT R

Less than 5 percent of invertebrate indicators of species composition were correlated ($p<0.05$) with poultry house density during summer 2006 and spring 2007 using both the all-site and low-urban datasets and by using regression analysis.

Relatively few indicators of species composition were related to stressors (DO, FGA cover, planktonic chlorophyll a, and TP, **Table 3.4**) during summer 2006. Only one metric was related to DO during summer 2006. Two to three indicators were related to TP in each dataset and from 2 to 6 indicators were related to benthic and planktonic algal biomass. In several cases, the predicted response was opposite of the usually predicted response for pollution. The percent of insects, percent of EPT individuals, and percent Ephemeroptera were positively correlated with algal biomass. This may be due to many of the Ephemeroptera being grazers or collectors that would use the algae as food sources and because some species can be relatively pollution tolerant.

Many indicators of species composition were related to stressors measured during summer 2007 (FGA cover, standard deviation in DO, maximum pH, and minimum DO, **Table 3.5**). With the full dataset, 11 to 12 of the indicators were significantly related with the predicted responses to indicators of FGA cover, the standard deviation in DO, and maximum pH. There is a very small probability that this many indicators would be significantly related with the predicted responses by chance. Few indicators were related to minimum DO or TP directly when all sites were included in the analysis. When restricting the dataset to only low urban influence sites, fewer indicators were correlated than when all sites were in the analysis. Five to six indicators were correlated with FGA cover, the standard deviation in DO, maximum pH, and TP concentration. Only one indicator was related to minimum DO.

3.3.2.2 Benthic invertebrates indicators of trophic structure

Less than 5 percent of invertebrate indicators of functional feeding groups (FFG) were related ($p<0.05$) with poultry house density during summer 2006 and spring 2007 using both the all-site and low-urban datasets and regression analysis.

Between zero and three FFG indicators were related to stressors during summer 2006 or spring 2007 (**Tables 3.6-3.7**). There was no consistent pattern for which stressor was most often related to FFG indicators during summer 2006. There was no consistent pattern for which FFG indicator was most often related to a stressor during summer 2006. During spring 2007, however, the predator and shredder indicators were respectively, negatively and positively correlated to FGA cover, the standard deviation in DO, and maximum pH using the full and low-urban datasets.

3.3.3 Discussion

Species composition of diatoms was clearly related to TP concentrations in IRW streams and indirectly related to poultry house operations in watersheds. Diatom species composition respond very sensitively to increasing nutrient concentrations as relative abundances of sensitive species decrease and species that require high nutrient concentration increase in relative abundance (Manyolov and Stevenson 2006). Diatom species composition responds to increases in P concentrations when TP is as low as 0.010 mg TP/L (Stevenson et. al in press).

The strongest effects of nutrient pollution on macrobenthic invertebrate communities were in spring 2007 compared to summer 2006. This may be due to the great accumulations of FGA and

EXHIBIT R

the occurrence of the most extensive growths of FGA during spring periods. Pollution tolerant organisms were positively related to FGA and related stressors (DO variation and high pH) during spring 2007. Pollution sensitive organisms were negatively related to FGA and related stressors (DO variation and high pH) during spring 2007. In addition, predators seemed to be reduced by FGA and related stressors (spring 2007). Shredders on the other hand, were positively related to FGA and related stressors. Shredders, which are often pollution sensitive, may have been using the FGA or material trapped in it as a food source.

Species composition of benthic macroinvertebrates was not related as well as diatoms to TP concentration in streams and resulting stressors. Other investigators have observed the same problem (Miltner and Rankin 1998; Wang et al. 2007). Trichoptera responded to nutrients during one season but not in the other. No macroinvertebrate indicators were correlated to nutrient pollution and related stressors consistently in both seasons. I have had similar experience with unpublished results in Michigan and Kentucky streams. This may be attributed to the potential variability in responses of species within orders, with some capitalizing on new conditions created by nutrient pollution and other species being eliminated. Most invertebrate metrics rely on similarly strong response of all species within higher taxonomic groups, like families and orders. In recent studies, I have been able to overcome difficulties of using metrics based on higher order of taxonomy by analysis with lower taxonomic levels and high resolution taxonomy.

The level of practical taxonomy is a big difference between diatom and invertebrate indicators based on species composition. Most invertebrate identifications are to the genus level and sometimes larvae are so young that only family or order can be identified. This creates a problem with metric use and loss of finer level information for invertebrates. State programs often use family level identifications in their stream assessments, whereas diatoms are almost always identified at the species level. This helps make response of routine diatom indicators to environmental change to be more precise and sensitive than routine macroinvertebrate indicators. Thus, our indicators may show a more sensitive response of diatoms to nutrient pollution, but unidentified species of macroinvertebrates may also be responding to nutrient pollution.

Section 4
Fish

4.1 Introduction

Fish are important elements of stream food webs (Hynes 1970, Allan and Castillo 2007), and they are elements of biodiversity valued by the public. Fish are used by many states of the US to assess the biological condition of streams and determine aquatic life use support. Biological assessment of state waters is mandated by the USEPA because of two goals of the Clean Water Act: protecting fish, shellfish, and wildlife; and restoring biological integrity. Nutrient criteria are being developed in many states based on finding of substantial effects of nutrients on the biological condition of fish assemblages (Miltner and Rankin 1998, Heiskary and Marcus 2003, Wang et al. 2007, Weigel and Robertson 2007).

The objectives of this section of the report are to document the injuries of fish species composition that are related to poultry house activities and nutrient pollution. I used the species composition of fish to calculate indicators of biological condition to measure injury. I hypothesize:

- The number of taxa and individuals sensitive to pollution will decrease with increasing poultry house density in watersheds, nutrient concentrations, and nutrient-related stressors in streams;
- The number of taxa and individuals that are tolerant to pollution will increase with increasing poultry house density in watersheds, nutrient concentrations, and nutrient-related stressors in streams.

4.2 Methods

Fish were collected during the summer of 2007 at 37 sites in the IRW. These sites were selected using a stratified random design with five strata defined by poultry house quintiles. Fish sampling protocols can be found in the Expert Witness Report from Darren Brown. Sampling and analysis of water chemistry follows methods described in section 2 and in the Expert Witness Report from Darren Brown.

Indicators of fish species composition were calculated with the same weighted average model as diatoms and invertebrates. Traits of taxa were characterized for their sensitivity or tolerance to pollution according to Dauwalter et al. (2003) and Barbour et al. (1999). Metrics and independent variables were transformed to reduce skewness and curtosis in the distribution to enable parametric statistical analyses. Multimetric indices of nutrient enrichment during the summer and spring stressors prior to summer sampling were calculated. Analysis was restricted to 22 sites with low percent urban land use and low influence from upstream wastewater treatment plants. Multiple linear regression was used to test the hypotheses fish diversity and species composition (indicated by metrics) would be affected by poultry house density or its stressors (DO, summer TP concentration, conditions indicated by a multimetric index of summer nutrient enrichment, and poultry house density in the watersheds) if watershed area was included in the models. Outliers in the data analysis were removed.

EXHIBIT R

The magnitude of effects of poultry house operations on metrics of fish assemblages was determined using the regression models resulting from the multiple linear regression analysis with watershed area and either poultry house density in watersheds. Effects were determined for the median watershed size, so half of the watersheds would be smaller and half larger than the watershed modeled. Predicted metric values were calculated separately for poultry house densities equal to zero and 8.235, which were the minimum and maximum poultry house densities in watersheds studied. The effects of poultry house activities were then calculated as the differences between metric values predicted when the minimum and maximum poultry house densities occurred in watersheds. The standardized effects were calculated as percentages by dividing the effect (difference between metric values predicted when the minimum and maximum poultry house densities occurred in watersheds) by model-predicted condition when poultry house density was equal to zero and multiplying that dividend by 100.

I evaluated the differences in the effect of poultry house density on fish metrics that could be caused by retaining non-significant coefficients for the constant and watershed area in the regression model. Regression models for relationships between fish metrics and either poultry house density or the multimetric index of summer enrichment were re-calculated by dropping constant and watershed area model coefficients if they were not statistically significant. The re-calculated models did not perform better than full models. The re-calculated models with the constant removed had problems with correlations between residuals and predicted values, which is a violation of the assumptions of the use of regression statistics. Dropping the watershed area factor from the model did not improve model performance and had little effect on effects. Therefore, effects of poultry house activities were calculated with full models

$$\text{Fish metric} = \text{constant} + \beta_1 * \ln(\text{watershed area}(mi^2)) + \beta_2 * \ln(\text{poultry houses}/mi^2)$$

Correlations between relative abundances of fish and good indicators of stress were calculated to determine which of the most common species were changing in relative abundance in association with nutrient enrichment. The summer multimetric nutrient index was used because it was most related to other stressors during the summer. Taxa with 7 or more observations at the 37 sites were selected for correlation with the summer multimetric nutrient index. Simple Pearson correlations were used to evaluate pattern. These analyses were supplemented with interpretations of graphs for the most highly correlated taxa.

The statistical methods used for evaluating the overall statistical significance of my analysis and individual tests are conservative. Additional injuries may be identified through the use of non-linear and multivariate statistical analysis. My opinion is based on the information that I have to date of submission of this report. I reserve the right to supplement my opinion based on review of additional data analyses and new information.

4.3 Results and Discussion

4.3.1 Land use and water chemistry

Finding: Indicators of nutrient enrichment were related to poultry house density in watersheds during summer 2007.

EXHIBIT R

In the dataset of 22 streams with low influence and land use associated with urban activity, poultry house density ranged from 0 to 8.2 houses/mi^2 in watersheds plus the 2 mile border upstream from sampling sites. Urban land use varied from 3 to 9 percent of the land use upstream in watersheds. Over this large range of poultry house density and small range in urban land use, there is still some relation between poultry house density and urban land use (r^2=0.314).

During the summer 2007 fish sampling period, DO varied from 3.8 to 12.55 with a mean of 7.8 mg/L in the 22 streams with low urban influence. Summer 2007 TP concentrations varied from 0.007 to 0.193 with a mean of 0.062. Summer 2007 TP concentrations were related significantly to poultry house density in watersheds (p<0.001, r^2=0.424), but summer 2007 DO concentrations were not (p=0.581). The multimetric nutrient index for summer, varying from -2.035 to 0.715 with a mean of -0.340, was highly related to poultry house density (p<0.001, r^2=0.659). Percent urban land use was also related to summer TP concentrations and the summer multimetric indicator of nutrient enrichment (SMMNI) (r^2=0.270 and r^2=0.314, respectively). The nutrient indicators were more precisely related to poultry house density than urban land use.

4.3.2 Fish species composition

Finding: Fish assemblages responded predictably to nutrient enrichment associated with poultry house activities.

Fish indicators were commonly related to summer nutrient enrichment and poultry house density in watersheds if watershed size was accounted for (p<0.05 with one-tailed test, **Table 4.1, Appendix 4.1**). Watershed size affected many fish indicators, as did nutrient enrichment associated with poultry house activities. DO was the only stressor to which no indicators were related, thus these results are not shown and will not be presented further. The number of coefficients with signs in the predicted direction was unlikely to occur by chance. On average, 11 of 13 fish assemblage indicators were related in the predicted direction to either TP, the summer multimetric nutrient index, poultry house density, or the multimetric index of springtime stressors (Table 4.1). Thus, the sign of the coefficient for the PHD-related stressor in the multiple regression model was the same as the predicted response 11 of 13 times. The probability of 11 of 13 relationships being in the predicted direction by chance was only 1 in 100 times assuming all analyses were independent and an even probability of either a positive or negative response. This is analogous to predicting the results of flipping a coin correctly 11 of 13 times. To test the sensitivity of the likelihood of getting 11 of 13 relationships as predicted to assumptions, we can vary the number of tests and the correct number of tests. If we assume that only half of the tests were independent from the others, and approximately the same ratio of correct to incorrect predictions, we could assume that we had only 5 independent analyses and 4 of the 5 were as predicted. The probablity of getting either 4 or 5 of 5 predictions correct, would be 19 in 100 times if we assume a 50/50 chance of either a positive or negative effect.

4.3.2.1 Fish species richness

Finding: Fish diversity decreased with nutrient enrichment associated with poultry house activities.

The number of fish species in streams averaged 14 and varied from 5 to 25 in the 22 IRW streams with low urban influence. The number of fish taxa was significantly related to the multimetric nutrient index (p<0.032), poorly related to poultry house density (p=0.140), and not related well to

EXHIBIT R

TP (($p<0.278$), DO ($p<0.628$), or multimetric indicator of springtime stressors ($p=0.638$). The effects of nutrient enrichment on fish density can be evaluated with the relationship:

$$\text{fish species number} = 3.139 + 3.378*\ln(\text{areami}^2) - 1.675*\text{SMMNI}$$

Where areami^2 is the area of the watershed and SMMNI is the summer multimetric nutrient index. The r^2 of this model is 0.646, indicating that 64.6 percent of the variation in fish species number is explained by the constant, watershed area, and the summer multimetric nutrient index (SMMNI). Over the range of the SMMNI, from -2.035 to 0.715, fish richness decreased from 16.43 species predicted when SMMNI=-2.035 and 11.82 when SMMNI was 0.715. This predicted decrease in fish richness with SMMNI is a 28 percent drop in fish species numbers according to predictions over the range of the SMMNI modeled.

4.3.2.2 Fish species composition responses

Finding: Many indicators of fish species composition were related to poultry house density and nutrient enrichment.

In addition to the number of fish species, poultry house density and nutrient enrichment were related to other indicators, which help characterize changes in fish species composition in streams (Table 4.1, Appendix 4.1). The following indicators were related significantly ($p<0.10$) to either poultry house density and the multimetric index of summer nutrient enrichment or both: number of sensitive taxa, proportion of sensitive individuals, number of predacious taxa, number of invertebrate-eating taxa, proportion of invertebrate eating individuals, the number of benthic taxa, and the number of lithophilic taxa (**Figure 4.1**). The following fish indicators were not related significantly to poultry house density or the multimetric index of summer nutrient enrichment: proportion of tolerant individuals, proportion stonerollers, the proportion of centrarchid individuals, and the proportion of benthic individuals.

Finding: Species composition of fish assemblages changed substantially in response to poultry house densities and nutrient enrichment.

The average change in fish indicators were about 20 percent from natural conditions across the range of poultry house activities in the IRW that was studied. Results in this paragraph were generated with models that included watershed area in all analyses, whether it was statistically significant or not. In addition, I will only include metric relationships with the SMMNI and poultry house density (not TP models) because of the greater probability of relationships between indicators and these independent (causal) variables. Effects ranged from a low of 0 percent for the effect of SMMNI on proportion centrarchid individuals compared to the high 74.7 percent reduction in the number of sensitive fish individuals in the streams that was related to poultry house density (**Tables 4.2 & 4.3**). In the latter example, we would expect to find 10.9 percent of the fish in a median sized IRW stream to be members of species that are considered to be sensitive to pollution when no poultry houses were in watersheds. In streams with the highest number of poultry houses that we sampled (0-8.2 houses/mi^2), we would expect 2.8 percent of the fish to be members of a sensitive species. Although the magnitude of species composition changes with nutrient pollution varied among indicators, the average change in indicators was approximately 20 percent. Twenty percent is an often recognized threshold for ecologically significant effects

EXHIBIT R

(Suter et al. 2000). Use of the 25 percent effect size as a threshold is also recommended for effluent toxicity testing (e.g., Klemm et al. 1994).

Twenty-five fish taxa were observed in 7 or more sites. Four taxa were significantly related to the summer multimetric nutrient index (Table 4.3). The orangethroat darter, slender madtom, and stippled darter were negatively and significantly ($p<0.05$) correlated with SUMNUTEMMI. The logperch was the only taxon negatively and significantly ($p<0.05$) correlated with the SUMNUTEMMI. The two taxa that were most negatively correlated with SUMNUTEMMI were the smallmouth bass and cardinal shiner. This statistical analysis was challenged by the large number of sites in which these species were not observed (**Figure 4.2**). Comparisons of predicted response based on literature traits to observed response with correlations were mixed. Traits of Barbour et al. (1999) for our six taxa with the greatest responses were not discriminating, i.e. most were moderate tolerance. The intolerance of these six taxa according to Dauwalter et al. (2003) had two taxa indicated as intolerant and one tolerant for the 3 taxa that were positively correlated with SUMNUTEMMI and two taxa indicated as intolerant and one tolerant for the 3 taxa that were negatively correlated with SUMNUTEMMI. Further analysis may provide more insight into the certainty of patterns reported.

4.3.3 Discussion

These results show that poultry house activities and contaminants associated with their operations are injuring fish species composition. The 20 percent loss of most attributes across the range of poultry house densities is significant. Dauwalter et al. (2003) evaluated the relative contribution of 44 environmental (physicochemical, water quality, and land use) variables on their Index of Biotic Integrity (IBI) for Ozark highland streams in Arkansas. Their regional IBI included a large number of fish community metrics, including those selected for use in this evaluation. P variables (as well as sulfate and chloride concentrations) were strongly correlated for the principal component analysis (PCA) category I, which accounted for over 41 percent of the variance in the fish IBI (which in turn consists of fish assemblage metrics). The authors of this study revealed that all the water quality data were collected during base-flow conditions, and that nutrient inputs into the waters are likely to occur mostly during episodic storm events. Therefore, the substantial contribution of P-related inputs on the fish community (as measured using the IBI) is probably underestimated in the Dauwalter et al. (2003) study.

Teasing out casual mechanisms is challenging when so many weather related factors cause variability in stream conditions. However, there is strong evidence for relations among poultry house operations, nutrient indicators, and DO during summer 2006 and 2007. The strong relationships between fish metrics and the SMMNI also showed that nutrients, most likely TP, caused injury to fish assemblages. The observation of the DO relationship with poultry houses during summer 2006 was probably because we sampled during early morning. DO concentrations vary greatly during the day and often more when nutrient stimulated-algae are particularly active. The sampling crews for fish measured dissolved oxygen whenever they go to the habitat to sample fish. They did not plan early morning sampling as the water chemistry sampling crew for summer 2006. Chronic effects of DO on fish are important. Low DO impairs motility of fish (e.g. Hill et al. 1978) and other physiological responses as well. Thus, multiple lines of evidence indicate poultry houses cause substantial injury to fish species composition.

EXHIBIT R

Section 5
Historical and Future Injury

5.1 Introduction and Methods

An objective of this study was to estimate reductions in TP concentrations that could be expected in the IRW under different management scenarios and relate them to the change in percent of the IRW watershed that would be injured for aesthetics and fish species composition under each scenario. In addition, I evaluate the likely differences in injury related to historic conditions in the watershed.

Historic and future P loads in the IRW were predicted using processed-based watershed models in the Expert Witness Report of Dr. Bernard Engel. TP loads in the Illinois River at Tahlequah, Baron Fork, and Caney Creek were predicted in one historic and 4 future scenarios. These are three major branches within the IRW and vary considerably in size. Historic conditions were reconstructed from 1950 to 1999. Four future scenarios were simulated for at least 50 years:

- Control – no change in management practices;
- No litter application in the future;
- No litter application and development of riparian buffer strips; and
- Growth based on the recent history of activities by the poultry industry.

The predicted discharge (cfs) and P loads (kg/d) simulated in the future scenarios were converted into TP concentrations. Engel includes the historic P concentrations in the Illinois River at Tahlequah, Baron Fork, and Caney Creek in his report.

I compared the simulated change in TP concentrations during the last 50 years and over the future 50 years to current conditions in the IRW to determine the change in percent of streams that are injured by P pollution. First, I selected two benchmarks for injury by P. The TP threshold for FGA cover, 0.027 mg TP/L, was selected as a benchmark for TP above which considerable risk of injury to aesthetics occurs. This benchmark has considerable support from observations in other studies as well (Dodds et al. 1997, Stevenson et al. 2006) in which 0.030 mg TP/L was identified as a benchmark for nutrient criteria. This benchmark was applied to spring TP conditions when nuisance FGA blooms occur. I also selected the TP thresholds at which at least three studies have found substantial evidence that fish communities are injured, 0.06 mg TP/L (Miltner and Rankin 1998, Wang et al. 1997). This benchmark was applied to summer conditions when we found evidence of fish responses to poultry house activities and nutrients. Thus, spring model data (March 15 thru June 15) were used to assess aesthetics injury associated with nuisance blooms of FGA cover. This is the most likely period for FGA blooms. Summer model data was selected from June 16 thru September 15 to characterize annual summer TP conditions that could cause injury to fish species composition.

EXHIBIT R

Simulations of TP concentration varied considerably over time because they included daily, seasonal, interannual, and long-term variability in weather conditions. To determine the central tendency of the changes in TP concentration in the four management scenarios, three locations, and two seasons, I used linear regression models. Thus, 24 linear regression models were developed for future scenarios.

Changes in modeled TP were relatively linear over time. However, variation around the line often increased under certain conditions. Further study of that variation phenomenon was beyond the scope of this study and did not interfere with the multi-year estimates of TP change needed for this study.

The 12 regression models for the four future scenarios, two seasons, and three IRW locations were used to predict the average change in TP concentrations from 2008 to 2058. Their general form was

$$TP_{s\text{-}avg} = \alpha + \beta\,(year*10000)$$

Here $TP_{s\text{-}avg}$ was the average TP concentration of the predicted daily concentration with the watershed models for either spring or summer. The regression models had to be calculated with year divided by 10,000 because the coefficients were so small that they appeared as zeros in the output if year was not transformed. This is easily corrected in the calculation of TP concentration. I did not log-transform TP concentration because it was more important to get an accurate prediction of the percent reduction in TP than to determine statistical significance of the relationship. The regression models were used to calculate expected TP concentration in either spring or summer of both 2008 and 2058. The percent change in TP concentration from 2008 to 2058 was determined for each of the 12 season-site-scenario conditions. The average percent change for each site-scenario combination was calculated with the 3 predicted changes for the three sites in the IRW. This was justified despite the great variability among predicted changes because the differences among sites were relatively systematic.

The average percent change for each site scenario combination was used to calculate changes in TP concentrations in the 96 subwatersheds that were sampled during summer 2006. These 96 watersheds were part of a pool of the 336 3rd order subwatersheds that were delineated and characterized for land use. The 96 subwatersheds were the subset was accessible by road for sampling. Details about the sampling and results can be found in the Expert Witness Report by Roger Olsen. The percent change for each of the four spring and four summer scenarios was applied to the TP concentration of each watershed to calculate the average TP concentration expected in a subwatershed in 2058.

The change in percent of the IRW watersheds that was injured under the four management scenarios was determined by ranking the sites by their TP concentrations in 2008 and the TP injury benchmarks (0.027 mg TP/L for algal blooms and aesthetics and 0.060 mg TP/L for fish species composition). All 96 subwatersheds were ranked by TP concentration in a table. The percentile of the site with the lowest TP concentration that exceeded the TP injury benchmarks was determined from the table. For example, if we ranked 100 sites with TP concentrations ranging from 1 to 100, and each was successively higher (e.g. 1, 2, 3 … 100), the 40 percent of the sites would have a TP concentration greater than 60. Since TP concentrations were changed proportionally from 2008 to

EXHIBIT R

2058 with the percent change factors for the different scenarios, ranking of sites did not change under the difference scenarios.

5.2 Results and Discussion

5.2.1 Historic Conditions

Finding: Historic TP concentrations, as late as 1950, would not have supported the frequent nuisance accumulations of FGA observed today.

Historic P conditions in the Illinois River at Tahlequah during the spring and summer increased from a predicted concentration of less than 0.030 mg TP/L to between 0.100 and 0.120 mg TP/L in 1999 (Dr. Bernard Engel's Expert Witness Report). Summer P concentrations were predicted to be slightly lower than spring P concentrations. Predicted changes in P concentrations in the Baron Fork during the spring were less, from less than 0.010 mg TP/L in 1950 to about 0.120 mg TP/L in 1999. Results from the Caney Creek were not used because of dry periods during summer conditions.

The increases in nutrient concentrations in the Illinois River at Tahlequah indicate that nutrients were low enough that extensive FGA cover would have been rare in 1950. The risk of nuisance FGA cover that would alter aesthetics and habitat for biodiversity increased greatly from 1950 to 1999. Oklahoma State phosphorus criterion for aesthetics use was predicted to be exceeded by the late 1950s in the Illinois River at Tahlequah. The probability of extensive FGA is great when TP concentrations are as high as 0.100 mg/L (Stevenson et al. 2006). Aesthetics problems would not have been as great in the Baron Fork. However, local problems were likely where P loading was sufficient to increase nutrient concentrations in small streams, but not in the main branch of the Baron Fork where P would become diluted and processed biologically.

The increases in nutrient concentrations in the Illinois River at Tahlequah and the Baron Fork indicate that nutrients were low enough that little risk to fish species composition from nutrient pollution existed in 1950. However, in the Illinois River, the increase in P concentrations exceeded the benchmark for fish effects, 0.060 mg TP/L, during the early 1970s.

5.2.2 Future Scenarios

Land use was characterized in 332 of the 336 3rd order subwatersheds of the IRW. The median poultry house density in watersheds was 1.375 houses/mi^2, with a minimum of 0, maximum of 7.095, and 25th and 75th quartiles of 0.267 and 3.717 houses/mi^2 (**Figure 5.1**). The median of urban land use was 4.67 percent of subwatersheds, with a minimum of 0.375, maximum of 88.847, and 25th and 75th quartiles of 3.142 and 7.59 percent. The median of percent agricultural land use was 44.4 percent of watersheds, with a minimum of 0, a maximum of 88.0, and 25th and 75th quartiles of 23.5 and 59.6 percent. In summary, most of the 3rd order subwatersheds had less than 10 percent urban land use and low poultry house densities.

Finding: Eleven percent more streams in the IRW would be injured in 50 years if growth of the industry continues as modeled. However, if litter application were halted, as many as 19 percent fewer streams would be injured. This represents a 30 percent difference in the number of watersheds injured in 50 years depending upon the management practices.

EXHIBIT R

The regression models, summarizing Engel's processed-based watershed models, showed decreases in both spring and summer TP concentrations over the next 50 years in IRW streams under control, no litter, and no litter plus buffer scenarios (**Table 5.1**). TP concentrations were predicted to increase by 24 percent (spring and summer) if poultry activity growth continues at the recent pace. The percent reduction in TP concentrations was successively higher for the control (-12 percent), no litter plus buffer (-27 to -30 percent), and no litter (-33 to -36 percent) scenarios. Discussion of reasons for differences in reductions of average seasonal TP concentrations among scenarios is beyond the scope of this report.

The predicted changes in proportion of IRW subwatersheds injured due to P pollution ranged from an 11 percent increase to a 19 percent decrease (**Tables 5.2 & 5.3**). The growth scenario caused a small increase in percent of watersheds injured for spring aesthetics, from 83 to 86 percent having TP concentrations greater than 0.027 mg TP/L. However during the summer, the percent of watersheds with greater than 0.060 mg TP/L increased from 47 to 58 percent according to the growth scenario.

The management scenario which could show the greatest improvement in TP concentrations over the next 50 years was the no litter scenario, although the no litter with buffer scenario was similar (Tables 5.2 & 5.3). The no litter scenario could cause a reduction in percent subwatersheds injured for spring aesthetics from 83 percent in 2008 to 64 percent in 2058. The greatest percent reduction in summer injury for fish species composition was from 47 percent in 2008 to 32 percent in 2058 with the no litter scenario.

EXHIBIT R

Section 6
Summary and Opinions

Nutrient pollution of streams, particularly with P, is a substantial cause of injury to beneficial uses in the IRW. The application of poultry waste to lands surrounding IRW streams, poultry houses are a substantial source of P in these streams (Expert Witness Report of Dr. Bernard Engel). The findings described and supported in this report are based on extensive studies of IRW streams that were designed to evaluate and document the resulting harm/injury to IRW streams that has resulted from the disposal of poultry wastes and resulting nutrient pollution within the IRW.

Overall, our studies show that poultry house operations are related to the nutrient pollution and that pollution is causing extensive injury to aesthetic condition in the watershed and substantial injury to biodiversity. Our primary indicator of aesthetic conditions was the percent cover of stream bottoms by FGA. Our primary indicators of injury to biodiversity were measures of the species composition of algae, invertebrates, and fish. These are commonly recommended indicators of beneficial uses of streams (USEPA 2000). They are used by many state agencies for assessing biological condition of streams.

The relationship between poultry house operations and injury to stream aesthetics and biodiversity was established with direct and indirect relationships. In the following summary of findings, linkages in the causal pathway between poultry house operations, nutrient pollution, algal growth, water chemistry stressors, and species composition are clear. See Figure 1.3 for orientation to the causal pathway.

Measured concentrations of N and P were relatively high in the IRW compared to many other regions and they were positively related to poultry house density in watersheds. When nutrient concentrations limited algal growth in IRW streams, P was most likely the nutrient that most limited algal growth in streams because N was in higher concentration compared to P and algal demands for the two nutrients.

Cover of FGA was higher than many other regions studied. Seldom are streams observed with greater than 50 percent cover of the stream bottom covered with FGA. Twenty-five percent of the streams surveyed had more than 50 percent of the stream bottom covered with FGA during spring 2007. Algal biomass in IRW streams was related both to TP concentration in the streams and to poultry house operations in the watershed. Even if we limited our analysis to streams with low urban impact to make sure we were not confusing the sources of P in streams, algal biomass in the streams was related both to TP concentration in the streams and to poultry house operations in the watershed.

A threshold in response of FGA to different P concentrations was observed in IRW streams at 0.027 mg TP/L during spring 2007. Above this nutrient concentration, there was a high risk of observing 50 percent or more of the stream bottom covered with macroalgae. Similar threshold responses in stream algal biomass have been observed in other regions of the US. These threshold changes in biomass along P gradients are usually around 0.030 mg TP/L.

EXHIBIT R

DO concentrations in streams were commonly less than the Oklahoma standards for supporting beneficial uses for fish and wildlife propagation. Daily fluctuations in DO increased with increased algal biomass and TP concentration during spring 2007 as a result of great differences between photosynthesis during the day and not at night. During summer 2006 sampling, when samples were collected during early morning, DO concentrations were lower in streams with high TP concentrations.

Average and maximum pH in IRW streams was high compared to the 9.0 pH that is an Oklahoma regulatory criterion and benchmark for unacceptable biological effects. The high pH levels were observed in streams with relatively high TP concentration, high algal biomass, and high poultry house operations.

Species composition of diatoms was clearly related to TP concentrations in IRW streams and indirectly related to poultry house operations in watersheds. Species composition of benthic macroinvertebrates was not related well to TP concentration in streams when using the routine indicators of macroinvertebrate species composition. Similar contrasts in response of algae and benthic invertebrates are evident in the literature (e.g. Pan et al. 1996, Stevenson et al. in press, Miltner and Rankin 1998, Wang et al. 2007). Diatoms respond directly and positively to TP, whereas responses by invertebrates are indirect. It is possible that more detailed analyses of the species composition of invertebrates with newly developed methods would show their relationships with TP.

The diversity of fish decreased and their species composition changed in IRW streams in relation with nutrient pollution and poultry house activities. Indicators of fish species composition were negatively affected by high levels of poultry house operations. On average, these negative effects were 20 percent of reference conditions. Although no threshold effects of nutrient pollution on fish species composition were observed in our survey of IRW streams, threshold responses have been observed in other studies around 0.060 mg TP/L (Wang et al. 2007).

The 0.027 mg TP/L threshold observed in the IRW for FGA cover and the 0.060 mg TP/L benchmark were used to define injurious levels of TP and to evaluate changes in injury to IRW streams under different management scenarios. Historically, nutrient concentrations were low in the IRW and would not have caused these problems. Eleven percent more streams in the IRW would be injured in 50 years if growth of the industry continues as in the recent past. If litter application was halted, at the end of 50 years as many as 19 percent fewer streams would be injured for aesthetics by FGA. After 50 years, only 15 percent would recover from injury to fish species composition. Depending on management practices, these models predict an 11 percent stream loss and a 19 percent stream gain that could result in a 30 percent difference in the number of Illinois River Watersheds that will be injured in 50 years.

Nutrient pollution associated with poultry house operations degraded the biological condition of fish, invertebrates, and algae as well as aesthetics in the IRW. The observed effects violate Oklahoma water quality standards for anti-degradation, both numeric and narrative standards for beneficial uses for aesthetics and fish and wildlife propagation, and biological criteria.

EXHIBIT R

Contractual agreement and signature

I was paid $150/hr for my responsibilities as an expert witness.

Signed

R. Jan Stevenson

R. Jan Stevenson, Ph.D.
Professor of Zoology

Section 7
References

Allan, J. D., D. L. Erickson, and J. Fay. 1997. The influence of catchment land use on stream integrity across multiple spatial scales. Freshwater Biology 37:149-161.

Barbour, M.T., J. Gerritsen, and B.D. Snyder. 1999. Rapid Bioassessment Protocols for Use in Streams and Rivers, 2nd edition. EPA 841-B-99-002, Office of Water (WH-553), United States Environmental Protection Agency, Washington, D.C.

Biggs, B. J. F. 2000. Eutrophication of streams and rivers: dissolved nutrient- chlorophyll relationships for benthic algae. Journal of the North American Benthological Society 19:17-31.

Capone, T.A. and J.A. Kushlan. 1991. Fish community structure in dry-season stream pools. Ecology 72(3): 983-992.

Carlisle, D.M., M.R. Meador, S.R.M. II, and P.M. Ruhl. 2007. Estimation and application of indicator values for common macroinvertebrate genera and families of the United States. Ecological Indicators 7:22-33.

Courtney, L.A. and W.H. Clements. 1998. Effects of acidic pH on benthic macroinvertebrate communities in stream microcosms. Hydrobiologia 379: 135-145

Deegan, L. A., and B. J. Peterson. 1992. Whole-River Fertilization Stimulates Fish Production in an Arctic Tundra River. Canadian Journal of Fisheries and Aquatic Sciences 49:1890-1901.

Dodds, W. K., V. H. Smith, and B. Zander. 1997. Developing nutrient targets to control benthic chlorophyll levels in streams: A case study of the Clark Fork River. Water Research 31:1738-1750.

Dodds, W.K. and R.M. Oakes. 2004. A technique for establishing reference nutrient concentrations across watersheds affected by humans. Limnology and Oceanography: Methods 2:331-341

Dudley, T. L., S. D. Cooper, and N. Hemphill. 1986. Effects of macroalgae on a stream invertebrate community. Journal of the North American Benthological Society 5:93-106.

Feldman, R.S. and E.F. Connor. 1992. The relationship between pH and community structure of invertebrates in streams of the Shenandoah National Park, Virginia, U.S.A. Freshwater Biology 27(2): 261-276

Fritz, K.M. J.A. Tripe, and C.S. Guy. 2002. Recovery of three fish species to flood and seasonal drying tallgrass prairie stream. Transactions of the Kansas Academy Science 105: 209-218.

Gelwick, F. P. 2000. Grazer identity changes the spatial distribution of cascading trophic effects in stream pools. Oecologia 125:573-583.

EXHIBIT R

Hart, D. D. 1981. Foraging and resource patchiness: field experiments with a grazing stream insect. Oikos 37:46-52.

Hilsenhoff, W. L. 1988. Rapid field assessment of organic pollution with a family level biotic index. Journal of the North American Benthological Society 7:65-68.

Humphrey, K.P., and R.J. Stevenson. 1992. Responses of benthic algae to pulses in current and nutrients during simulations of subscouring spates. Journal of the North American Benthological Society 11:37-48.

Ishii, S., T. Yan, D. A. Shively, M. N. Byappanahalli, R. L. Whitman, and M. J. Sadowsky. 2006. *Cladophora* (Chlorophyta) spp. harbor human bacterial pathogens in nearshore water of Lake Michigan. Applied and Environmental Microbiology 72:4545-4553.

Johnson, L. B., C. Richards, G. E. Host, and J. W. Arthur. 1997. Landscape influences on water chemistry in Midwestern stream ecosystems. Freshwater Biology 37:193-208.

Karr, J. R., and E. W. Chu. 1999. Restoring life in running waters. Island Press, Washington, DC.

Klemm, D., G. Morrison, T. Norberg-King, W. Peltier, and M. Heber. 1994. Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Marine and Estuarine Organisms, 2nd ed. Office of Research and Development, U.S. Environmental Protection Agency, Cincinnati. EPA/600/4-91/003.

Klemm, D.J., K.A. Blocksom, W.T. Thoeny, F.A. Fulk, A.T. Herlihy, P.R. Kaufmann, and S.M. Cormier. 2002. Methods Development and Use of Macroinvertebrates as Indicators of Ecological Conditions for Streams in the Mid-Atlantic Highlands Region. Environmental Monitoring and Assessment 78:169-212

Kohler, S. L. 1985. Identification of stream drift mechanisms: an experimental and observational approach. Ecology 66:1749-1761.

Lemly, A. D. 1998. Bacterial growth on stream insects: potential for use in bioassessment. Journal of the North American Benthological Society 17:228-238.

Lohman, K., J. R. Jones, and B. D. Perkins. 1992. Effects of Nutrient Enrichment and Flood Frequency on Periphyton Biomass in Northern Ozark Streams. Canadian J. Fish. Aquat. Sci. 49:1198-1204.

Manoylov, K. M., and R. J. Stevenson. 2006. Density-dependent algal growth along N and P nutrient gradients in artificial streams. In:. Pages 333-352 *in* N. Ognjanova-Rumenova and K. Manoylov, editors. Advances in Phycological Studies. Pensoft Publishers., Moscow, Russia.

McCormick, P. V., and R. J. Stevenson. 1989. Effects of snail grazing on benthic algal community structure in different nutrient environments. Journal of the North American Benthological Society 82:162-172.

EXHIBIT R

Millennium Ecosystem Assessment. 2005. Ecosystems and Human Well-being: Synthesis. Island Press, Washington, DC.

OWRB. 2005. 2005 Report of the Oklahoma Beneficial Use Monitoring Program Streams Report. http://www.owrb.ok.gov/quality/monitoring/bump/pdf_bump/2005BUMPStreams-Report.pdf

Peterson, B. J., L. Deegan, J. Helfrich, J. Hobbie, M. Hullar, B. Moller, T. E. Ford, A. Hershey, A. Hiltner, G. Kipphut, M. A. Lock, D. M. Fiebig, V. McKinley, M. C. Miller, R. Vestal, R. Ventullo, and G. Volk. 1993. Biological responses of a tundra river to fertilization. Ecology 74:653-672.

Power, M. E., W. J. Matthews, and A. J. Stewart. 1985. Grazing minnows, piscivorous bass, and stream algae: dynamics of a strong interaction. Ecology 66:1448-1456.

Schlosser, I.J. 1982. Fish community structure and function along two habitat gradients in a headwater stream. Ecological Monographs 52: 395-414.

Stevenson, R. J., and R. Glover. 1993. Effects of Algal Density and Current on Ion-Transport through Periphyton Communities. Limnology and Oceanography 38:1276-1281.

Stevenson, R. J., R. C. Bailey, M. C. Harass, C. P. Hawkins, J. Alba-Tercedor, C. Couch, S. Dyer, F. A. Fulk, J. M. Harrington, C. T. Hunsaker, and R. K. Johnson. 2004. Interpreting results of ecological assessments. Pages 85-111 *in* M. T. Barbour, S. B. Norton, H. R. Preston, and K. W. Thornton, editors. Ecological Assessment of Aquatic Resources: Linking Science to Decision-Making. Society of Environmental Toxicology and Contamination Publication, Pensacola, Florida.

Stevenson, R.J. in press. Algae of river ecosystems. In: G. Likens. Editor in Chief. Encyclopedia of Inland Waters. Elsevier.

Suter, G. W. 1993. Ecological Risk Assessment. Lewis Publishers, Boca Raton, Florida.

Suter, G., R. Efroymson, B. Sample, and D. Jones. 2000. Ecological Risk Assessment for Contaminated Sites.Lewis, Boca Raton. 438 p.

USEPA. 2000. Nutrient criteria technical guidance manual: rivers and streams. EPA-822-B-00-002, United States Environmental Protection Agency, Washington, D.C.

USEPA. 2007. National Water Quality Inventory: Report to Congress. 2002 Reporting Cycle. EPA 841-R-07-001. United States Environmental Protection Agency, Washington, DC.

Valett, H. M., S.G. Fisher, N.B. Brimm, and P. Camill. 1994. Vertical hydrologic exchange and ecological stability of a desert stream ecosystem. Ecology 75:548-560.

Van Dam, H., A. Mertenes, and J. Sinkeldam. 1994. A coded checklist and ecological indicator values of freshwater diatoms from the Netherlands. Netherlands Journal of Aquatic Ecology 28:117-133.

EXHIBIT R

Vannote, R. L., G. W. Minshall, K. W. Cummins, J. R. Sedell, and C. E. Cushing. 1980. The River Continuum Concept. Canadian Journal of Fisheries and Aquatic Sciences 37:130-137.

Whitmore, C.M., C.E. Warren, and P. Doudoroff. 1960. Avoidance Reactions of Salmonid and Centrarchid Fishes to Low Oxygen Concentrations. Transactions of the American Fisheries Society. Volume 89, Issue 1.

EXHIBIT R

Appendix 4.1. Full fish models with a constant, β_1 being ln(watershed area(mi^2)) and β^2 being the variable in the Independent Variable column. The (p) designates the attained statistical significance of the constant or coefficients (β) in the model.

Dependent Variable	Indep Var	r2	constant	const(p)	B1	B1(p)	B2	B2(p)
Number of taxa	TP	0.570	0.647	0.847	3.515	0.000	-0.823	0.278
Number of sensitive taxa	TP	0.677	-1.829	0.084	1.382	0.000	-0.129	0.588
Percent sensitive individuals	TP	0.614	-0.248	0.045	0.152	0.000	-0.015	0.535
Percent tolerant individuals	TP	0.000	0.335	0.015	-0.016	0.528	0.021	0.478
Number of predacious taxa	TP	0.639	-0.783	0.771	3.071	0.000	-0.351	0.556
Percent stonerollers	TP	0.149	0.000	1.000	0.057	0.032	-0.013	0.666
Percent centrarchid individuals	TP	0.000	0.194	0.077	0.012	0.570	0.028	0.260
Number invert-eating taxa	TP	0.596	0.046	0.968	1.238	0.000	-0.220	0.405
Percent invert-eating individuals	TP	0.000	0.160	0.227	0.028	0.308	-0.005	0.866
Number of benthic taxa	TP	0.576	1.304	0.051	0.664	0.000	-0.297	0.055
Percent benthic individuals	TP	0.000	0.493	0.003	-0.034	0.278	0.007	0.835
Number of lithophilic taxa	TP	0.648	2.058	0.171	1.804	0.000	-0.325	0.327
Number of taxa	SMMNI	0.646	3.139	0.121	3.378	0.000	-1.675	0.032
Number of sensitive taxa	SMMNI	0.711	-1.475	0.026	1.358	0.000	-0.386	0.125
Percent sensitive individuals	SMMNI	0.669	-0.210	0.009	0.151	0.000	-0.045	0.080
Percent tolerant individuals	SMMNI	0.000	0.268	0.004	-0.015	0.573	0.019	0.557
Number of predacious taxa	SMMNI	0.711	-0.685	0.683	3.342	0.000	-1.620	0.017
Percent stonerollers	SMMNI	0.162	0.040	0.616	0.056	0.035	-0.022	0.497
Percent centrarchid individuals	SMMNI	0.000	0.101	0.158	0.013	0.556	0.000	0.991
Number invert-eating taxa	SMMNI	0.524	0.492	0.548	1.281	0.000	-0.514	0.087
Percent invert-eating individuals	SMMNI	0.053	0.243	0.015	0.004	0.881	-0.063	0.094
Number of benthic taxa	SMMNI	0.606	2.217	0.000	0.635	0.000	-0.370	0.026
Percent benthic individuals	SMMNI	0.042	0.177	0.043	0.024	0.370	-0.041	0.210
Number of lithophilic taxa	SMMNI	0.813	3.694	0.000	1.506	0.000	-0.855	0.002
Number of taxa	ln(PHD)	0.594	4.710	0.048	3.634	0.000	-1.556	0.140
Number of sensitive taxa	ln(PHD)	0.724	-0.936	0.162	1.441	0.000	-0.581	0.073

EXHIBIT R

Dependent Variable	Indep Var	r2	constant	const(p)	B1	B1(p)	B2	B2(p)
Percent sensitive individuals	ln(PHD)	0.705	-0.146	0.051	0.163	0.000	-0.074	0.024
Percent tolerant individuals	ln(PHD)	0.000	0.254	0.009	-0.017	0.517	0.014	0.738
Number of predacious taxa	ln(PHD)	0.661	0.885	0.649	3.596	0.000	-1.568	0.088
Percent stonerollers	ln(PHD)	0.143	0.037	0.661	0.055	0.042	0.011	0.801
Percent centrarchid individuals	ln(PHD)	0.000	0.088	0.249	0.011	0.613	0.016	0.650
Number invert-eating taxa	ln(PHD)	0.622	1.151	0.116	1.306	0.000	-0.520	0.176
Percent invert-eating individuals	ln(PHD)	0.000	0.302	0.008	0.014	0.648	-0.059	0.244
Number of benthic taxa	ln(PHD)	0.548	2.567	0.000	0.693	0.000	-0.349	0.119
Percent benthic individuals	ln(PHD)	0.020	0.216	0.027	0.032	0.234	-0.045	0.280
Number of lithophilic taxa	ln(PHD)	0.837	4.943	0.000	1.655	0.000	-1.208	0.001

EXHIBIT R

Table 2.1 Parameters measured during each field campaign and their category. The variable category determines their use in data analysis

Variable Name	Sampling Campaign			Variable Category
	Sum '06	Spr '07	Sum '07	
Streams Sampled	72	72	37	Na
Poultry house density (houses/mi2)	1	1	1	Land use and landscape
Percent urban land use	1	1	1	Land use and landscape
Percent agricultural land use	1	1	1	Land use and landscape
Watershed area (mi2)	1	1	1	Land use and landscape
Total phosphorus concentration (mg P/L)	1	8	1	Nutrient
Total nitrogen concentration (mg P/L)	1	1	1	Nutrient
Soluble reactive phosphorus concentration (mg P/L)	1	8	1	Nutrient
Total dissolved phosphorus concentration (mg P/L)	1	8	1	Nutrient
Nitrate-nitrite concentration (mg N/L)	1	1	1	Nutrient
Ammonia concentration (mg N/L)	1	1	1	Nutrient
Total organic carbon	1	1	1	Nutrient
Filamentous algal cover (percentage)	1	8	1	Algal biomass
Benthic algal biomass (µg chl a/cm2)	1	1	1	Algal biomass
Planktonic algal biomass (µg chl a/L)	1	1	1	Algal biomass
Dissolved oxygen concentration average (mg/L)	1	8	1	Stressor
Dissolved oxygen concentration minimum (mg/L)		1		Stressor
Dissolved oxygen concentration standard deviation (mg/L)		1		Stressor
pH average	1	8	1	Stressor
pH maximum		1		Stressor
pH standard deviation		1		Stressor
Conductivity (µS/cm)	1	8	1	Stressor
Temperature (°C)	1	8	1	Stressor
Turbidity	1	1	1	Stressor
Percent Canopy Cover	1	1	1	Land use and landscape
Diatom indicators (species composition)	1	1	1	Species Composition
Invertebrate indicators (species composition)	1	1	1	Species Composition
Fish indicators (species composition)			1	Species Composition

EXHIBIT R

Table 2.2. Correlations between algal biomass, nutrients, and land use. Correlations greater than 0.36 were significant with $p<0.05$ and a 2-tailed hypothesis test.

Independent Variable	FGA Cover	Ben. Algal Biomass	Plank. Algal Biomass
Ln (SRP)	0.476	0.553	0.457
Ln (TOC)	0.610	0.483	0.516
Ln (TDP)	0.499	0.566	0.483
Ln (TP)	0.536	0.587	0.558
Ln (NO3)	0.245	0.318	0.177
Ln (TN)	0.191	0.229	0.069
Agr. LU	0.332	0.319	0.384
Ln (PHD)	0.316	0.266	0.387
Ln (Urban LU)	0.352	0.480	0.354
Ln (Watershed Area (mi2))	0.081	0.164	0.440

EXHIBIT R

Table 3.1. Descriptions of diatom and invertebrate indicators in text and Tables 3.2-3.5. Also, reference sources (Ref) are provided for traits of the species where 1 is Stevenson et al (in press), 2 is van Dam et al. (1994), 3 is Hilsenhoff (1988), 4 is Klemm et al. (2002), 5 is Carlisle et al. (2007), and 6 is Barbour et al. (1999).

Indicator Code	Indicator Description (if needed for Acronyms)	Ref
Diatom Metrics		
MAIATSI	Mid-Atlantic Integrate Assessment Trophic Status Index	1
VDTSIWO7_10	Van Dam Trophic Status Index raised to the 10th power	2
VDOXY	Van Dam Oxygen Pollution Index	2
VDNHET	Van Dam Heterotrophy Index	2
VDSAP	Van Dam Saprobic Index	2
SPCTOLMAIA	Proportion of Tolerant Taxa according to MAIA TSI traits	1
SPCTOLVDSAP	Proportion of Tolerant Taxa according to VD TSI traits	2
SPCSENSMAIA	Proportion of Sensitive Taxa according to MAIA TSI traits	1
PCSENSVDSAP	Proportion of Sensitive Taxa according to VD TSI traits	2
Invertebrate Metrics		
IV_TAXANO	Invertebrate Taxa Number	
PCINSECTA_10	(Proportion of Invertebrates that were Insect)^10	
PCEPT	Proportion (Ephemeroptera+Trichoptera+Plecoptera)	
SQRPCEPT	sqr(Proportion (Ephemeroptera+Trichoptera+Plecoptera))	
SQR2TRICHOP	Sqr(sqr(proportion Trichoptera))	
PCEPHEM	Proportion Ephemeroptera	
SQRPCEPHEM	sqr(Proportion Ephemeroptera)	
SQRPCCHIRO	sqr(proportion Chironomids)	
PCCHIRO	Proportion Chironomids	
LHILSENHOF	log(Hilsenhoff Index)	3
HILSENHOF3	(Hilsenhoff Index)^3	3
IV_AVGPTV	Average Pollution Tolerance Value	4
IV_PTV	Pollution Tolerance Index	4
IV_IONS	Ion Tolerance Index	5
IV_NUTS	Nutrient Tolerance Index	5
IV_DOT	DO Stress Tolerance Index	5
IV_SS	Suspended Sediment Tolerance Index	5
IV_FINS	Percent Fines Tolerance Index	5
PCTOLPTV	Proportion Tolerant Individuals	4
PCSENSPTV	Proportion Sensitive Individuals	4
SQRPCVSENSPTV	Proportion very sensitive Individuals	4
PRTAXA	Number of Predator Taxa	6
SQRPROPPR	sqr(proportion predator individuals)	6
FCTAXA	Number of Filter Collector Taxa	6
SQRPROPFC	sqr(proportion of filter collector individuals)	6
GCTAXA	Number of Gather collector Taxa	6

EXHIBIT R

Indicator Code	Indicator Description (if needed for Acronyms)	Ref
PROPGC	Proportion of gatherer collector individuals	6
FCGCTAXA	Number of all Collector Taxa	6
PROPFCGC	Proportion of all collector taxa	6
SCTAXA	Number of scraper taxa	6
PROPSC	proportion of scraper taxa	6
SQRPROPSC	sqr(proportion of scraper taxa)	6
SHTAXA	Number of shredder taxa	6
SQRPROPSH	sqr(proportion of shredder taxa)	6
SQR2PROPSH	sqr(sqr(proportion of shredder taxa))	6

Table 3.2. Expected responses (ER) of diatom metrics to total phosphorus concentration (TP) and poultry house density (PHD). Correlation coefficients (*r*) are shown, which vary from -1.0 to 1.0 depending upon the precision of relationships between metrics and the independent variables. Correlation coefficients shown in bold are statistically significant with a one-tail test based on ER and a *p* value <0.05 after Bonferroni correction for multiple tests.

Variable	ER	Summer 2006		Spring 2007	
		LNTP	LCHDPMI2	LNTP	LCHDPMI2
MAIATSI	+	**0.538**	0.119	**0.714**	0.264
VDTSIWO7_10	+	0.294	-0.112	**0.450**	0.151
VDOXY	+	**0.593**	0.034	**0.674**	**0.378**
VDNHET	+	**0.568**	-0.001	**0.723**	0.291
VDSAP	+	0.369	0.185	**0.419**	0.226
SPCTOLMAIA	+	**0.546**	0.025	**0.642**	0.134
SPCTOLVDSAP	+	0.309	0.035	**0.392**	**0.363**
SPCSENSMAIA	-	**-0.509**	-0.206	**-0.636**	-0.308
PCSENSVDSAP	-	**-0.516**	-0.169	**-0.493**	-0.199

EXHIBIT R

Table 3.3. Relationships between poultry house density and diatom indicators of species composition during summer 2006 and spring 2007. The proportion of variation in diatom indicators that can be explained by models that included both poultry house density and percent urban land use in watersheds is indicated by r^2. The Coef and AS are the coefficient and the attained statistical significance (p) of the coefficient in the models. Models were run separately for summer 2006 and spring 2007. ER is the expected response in a metric with increasing nutrient pollution.

Metric	ER	Summer 2006			Summer 2007		
		r2	Coef	AS	r2	Coef	AS
MAIATSI	+	0.095	0.328	0.079	0.379	0.415	0.001
VDTSIWO7_10	+	0.000	-194493	0.730	0.200	710584 1	0.073
VDOXY	+	0.007	0.060	0.621	0.292	0.350	0.000
VDNHET	+	0.011	0.026	0.752	0.269	0.167	0.002
VDSAP	+	0.014	0.164	0.100	0.088	0.118	0.032
SPCTOLMAIA	+	0.064	0.041	0.366	0.362	0.079	0.029
SPCTOLVDSAP	+	0.000	0.015	0.698	0.113	0.086	0.002
SPCSENSMAIA	-	0.084	-0.109	0.027	0.189	-0.125	0.002
PCSENSVDSAP	-	0.010	-0.064	0.139	0.211	-0.075	0.026

EXHIBIT R

Table 3.4 Correlations between invertebrate indicators of species composition and pollution tolerance and stressors during summer 2006. The correlation coefficients in bold were statistically significant. Remember low DO is stressor, so expect opposite metric response. LN1CLADCOV is the ln(percent FGA cover+1), LNCHL_UGL is the ln(planktonic chl a (µg chl a/L), LNTP is the ln(TP (mg/L)). ER means expected response.

	ER	All Sites					Low Urban Sites				
		DO (mg/L)	LN1CLADCOV	LNCHL_UGL	ug Chl a/cm^2	LNTP	DO (mg/L)	LN1CLADCOV	LNCHL_UGL	ug Chl a/cm^2	LNTP
IV_TAXANO	-	-0.165	0.141	-0.016	-0.002	0.007	0.031	0.264	0.021	***0.385***	0.249
PCINSECTA_10	-	0.011	***0.528***	0.000	-0.156	-0.038	0.283	***0.620***	-0.071	***-0.330***	***-0.335***
PCEPT	-	0.092	***0.235***	-0.073	-0.028	***0.224***	0.079	0.094	-0.097	-0.242	-0.027
SQR2TRICHOP	-	0.067	-0.087	***-0.289***	-0.028	0.140	0.045	-0.210	***-0.605***	0.016	-0.053
PCEPHEM	-	0.008	***0.377***	0.121	0.036	0.150	0.004	0.274	0.247	-0.149	0.051
SQRPCCHIRO	+	0.009	0.178	0.132	0.031	-0.133	0.257	***0.451***	-0.156	0.133	-0.260
LHILSENHOF	+	0.065	0.156	***0.398***	0.108	0.055	0.249	***0.405***	0.299	0.192	0.218
IV_AVGPTV	+	0.041	-0.133	***0.315***	0.007	-0.134	-0.041	0.048	***0.556***	0.183	0.231
IV_PTV	+	-0.052	***-0.396***	0.121	0.013	-0.127	-0.036	-0.244	0.116	0.180	0.120
IV_IONS	+	0.093	-0.178	-0.024	0.174	0.057	0.255	-0.229	-0.220	***0.553***	0.290
IV_NUTS	+	0.093	-0.178	-0.024	0.174	0.057	0.255	-0.229	-0.220	***0.553***	0.290
IV_DOT	+	-0.106	0.154	***0.322***	0.009	***0.216***	-0.205	***0.432***	***0.390***	***-0.419***	0.165
IV_SS	+	-0.010	0.154	0.087	***0.276***	0.177	-0.140	0.204	-0.059	0.277	0.092
IV_FINS	+	0.064	-0.083	***0.199***	***0.236***	0.162	-0.012	-0.053	-0.036	***0.366***	***0.400***
PCTOLPTV	+	-0.014	***-0.293***	0.060	0.079	-0.023	-0.058	-0.200	0.027	0.310	0.145
PCSENSPTV	-	0.047	***0.340***	0.013	0.013	0.129	0.054	0.175	0.057	-0.060	0.047
SORPCVSENSPTV	-	***0.252***	0.206	-0.191	0.203	***0.263***	0.346	0.074	-0.359	0.193	-0.047

EXHIBIT R

Table 3.5 Correlations between invertebrate indicators of species composition and pollution tolerance and stressors during spring 2007. The correlation coefficients in bold were statistically significant. Remember low DO is stressor, so expect opposite metric response. SPCGREENCOV is the square root of the percent of FGA cover, STDEVDO is the standard deviation in DO, MAXPH is the maximum pH, and MINDO is the minimum DO.

Metric	ER	All Sites					Low Urban Sites				
		SPCGREENCOV	STDEVDO	MAXPH	MINDO	TP (mg/L)	SPCGREENCOV	STDEVDO	MAXPH	MINDO	TP (mg/L)
IV_TAXANO	-	-0.091	-0.066	-0.079	-0.074	0.062	-0.110	-0.028	0.087	-0.134	-0.111
PCINSECTA_10	-	0.047	0.069	0.160	0.008	0.000	0.028	0.121	0.303	-0.120	0.252
SQRPCEPT	-	**-0.322**	**-0.291**	**-0.288**	0.068	-0.096	-0.213	-0.121	0.112	0.109	-0.112
SQR2TRICHOP	-	**-0.242**	-0.144	**-0.263**	-0.009	-0.136	**-0.428**	-0.264	**-0.315**	0.034	**-0.329**
SQRPCEPHEM	-	-0.136	-0.109	-0.164	0.006	0.015	-0.032	-0.058	0.195	0.092	0.047
PCCHIRO	+	**0.254**	**0.254**	**0.379**	-0.084	0.002	0.230	0.261	0.253	**-0.344**	**0.294**
HILSENHOF3	+	**0.312**	**0.211**	**0.205**	0.007	0.029	0.244	0.050	-0.064	0.002	0.095
IV_AVGPTV	+	**0.233**	**0.306**	**0.355**	-0.094	0.055	0.054	0.188	0.074	-0.117	0.036
IV_PTV	+	**0.311**	**0.225**	0.188	-0.010	0.049	0.167	0.093	-0.169	-0.024	0.034
IV_IONS	+	**0.318**	**0.309**	**0.364**	-0.010	-0.111	**0.458**	**0.475**	**0.380**	-0.205	**0.431**
IV_NUTS	+	**0.318**	**0.309**	**0.364**	-0.010	-0.111	**0.458**	**0.475**	**0.380**	-0.205	**0.431**
IV_DOT	+	-0.022	0.028	0.164	-0.128	0.103	-0.136	-0.067	-0.001	-0.227	-0.028
IV_SS	+	**0.229**	**0.266**	**0.469**	-0.044	-0.086	**0.403**	0.273	**0.506**	-0.118	**0.397**
IV_FINS	+	**0.285**	**0.254**	**0.336**	-0.027	-0.155	**0.433**	**0.377**	**0.309**	-0.199	**0.344**
PCTOLPTV	+	**0.304**	**0.317**	**0.280**	-0.092	-0.013	0.223	0.207	0.003	-0.213	0.203
PCSENSPTV	-	**-0.377**	**-0.330**	**-0.205**	0.092	-0.161	-0.209	-0.189	0.173	0.155	-0.068
SQRPCVSENSPTV	-	-0.182	-0.126	-0.158	0.113	-0.042	-0.149	-0.040	-0.001	0.049	0.041

EXHIBIT R

Table 3.6 Correlations between invertebrate indicators for functional feeding groups and stressor during summer 2006. The correlation coefficients in bold were statistically significant. Remember low DO is stressor, so expect opposite metric response. LN1CLADCOV is the ln(percent FGA cover+1), LNCHL_UGL is the ln(planktonic chl a (μg chl a/L), LNTP is the ln(TP (mg/L)).

	ER	All Sites					Low Urban Sites				
		DO (mg/L)	LN1CLADCOV	LNCHL_UGL	ug Chl a/cm^2	LNTP	DO (mg/L)	LN1CLADCOV	LNCHL_UGL	ug Chl a/cm^2	LNTP
PRTAXA	-	***-0.305***	0.138	0.083	-0.118	***-0.382***	-0.112	0.194	0.260	0.043	-0.131
SQRPROPPR	-	-0.088	0.120	***0.215***	0.176	-0.200	-0.010	0.188	0.233	0.068	-0.162
FCTAXA	+	-0.050	0.025	-0.120	0.074	0.300	-0.157	0.076	-0.295	0.039	0.258
SQRPROPFC	+	0.075	-0.087	***-0.272***	0.004	0.141	0.107	-0.073	***-0.557***	-0.095	-0.180
GCTAXA	+	0.144	0.162	-0.023	0.093	0.020	***0.411***	0.213	-0.065	***0.359***	0.111
PROPGC	+	0.003	***0.198***	0.153	0.061	0.051	0.091	-0.015	0.205	0.021	0.209
FCGCTAXA	+	0.105	0.154	-0.073	0.114	0.151	***0.345***	0.243	-0.183	***0.373***	0.219
PROPFCGC	+	0.108	0.100	-0.026	0.016	0.175	0.209	-0.152	-0.154	-0.112	0.124
SCTAXA	+	-0.099	-0.011	0.019	-0.027	***0.326***	***-0.327***	-0.014	0.254	0.187	***0.358***
PROPSC	+	-0.020	-0.134	-0.054	-0.035	-0.066	-0.161	0.049	0.123	0.015	-0.003
SHTAXA	+	-0.116	0.143	-0.115	-0.110	-0.174	0.088	***0.465***	-0.273	-0.067	-0.142
SQR2PROPSH	+	-0.123	0.158	-0.063	-0.013	-0.061	0.005	0.394	-0.211	0.082	-0.041

EXHIBIT R

Table 3.7 Correlations between invertebrate indicators of functional feeding groups and stressors during spring 2007. The correlation coefficients in bold were statistically significant. Remember low DO is stressor, so expect opposite metric response. SPCGREENCOV is the square root of the percent of FGA cover, STDEVDO is the standard deviation in DO, MAXPH is the maximum pH, and MINDO is the minimum DO. ER mean expected response.

Metric	ER	All Sites					Low Urban Sites				
		SPCGREENCOV	STDEVDO	MAXPH	MINDO	TP (mg/L)	SPCGREENCOV	STDEVDO	MAXPH	MINDO	TP (mg/L)
PRTAXA	-	***-0.397***	***-0.367***	***-0.281***	0.008	-0.190	***-0.453***	***-0.357***	-0.133	0.049	***-0.468***
SQRPROPPR	-	***-0.359***	***-0.378***	***-0.305***	0.120	-0.186	-0.176	-0.130	-0.041	0.148	-0.286
FCTAXA	+	0.055	0.133	0.023	-0.015	0.039	-0.197	0.000	0.108	-0.005	-0.064
SQRPROPFC	+	0.187	0.116	-0.158	0.000	-0.030	0.099	0.180	0.041	0.005	0.054
GCTAXA	+	-0.002	0.039	0.010	-0.088	0.160	-0.026	0.108	0.239	-0.098	0.010
PROPGC	+	-0.121	-0.186	-0.045	0.092	-0.065	-0.159	-0.233	-0.131	0.069	-0.199
FCGCTAXA	+	0.020	0.083	0.017	-0.074	0.140	-0.103	0.087	0.236	-0.080	-0.019
PROPFCGC	+	-0.010	-0.141	-0.174	0.122	-0.092	-0.132	-0.135	-0.123	0.122	-0.229
SCTAXA	+	-0.062	-0.187	-0.229	0.005	0.063	0.014	-0.016	-0.125	-0.181	-0.060
SQRPROPSC	+	-0.194	-0.214	-0.150	-0.084	0.188	-0.268	-0.237	0.014	0.005	-0.175
SHTAXA	+	***0.260***	0.110	0.181	0.035	0.070	0.233	0.029	0.166	0.037	0.168
SQRPROPSH	+	***0.409***	***0.412***	***0.326***	-0.131	0.071	***0.413***	***0.415***	0.194	***-0.325***	***0.514***

EXHIBIT R

Table 4.1 Fish responses to nutrients, and poultry house density stressor indicators are indicated by the coefficients (Coeff) from regression models that also included ln(watershed area(mi^2)). The coefficients for watershed area are not shown. ER indicates the expected response and p indicates the attained statistical significance of the regression coefficient. The independent variables in the model are TP, summer multimetric nutrient index (SMMNI), poultry house density (PHD), and a multimetric stressor using spring 2007 DO and pH conditions. See Appendix 4.1 for the full models.

Indicator	ER	f(TP)		f(SMMNI)		f(ln(PHD))		f(sprDO-pH)	
		Coeff	p	Coeff	P	Coeff	p	Coeff	p
Number of taxa	-	-0.823	0.278	-1.675	**0.032**	-1.556	0.140	-0.613	0.638
Number of sensitive taxa	-	-0.129	0.588	-0.386	0.125	-0.581	**0.073**	-0.329	0.333
Proportion sensitive individuals	-	-0.015	0.535	-0.045	**0.080**	-0.074	**0.024**	-0.007	0.839
Proportion tolerant individuals	+	0.021	0.478	0.019	0.557	0.014	0.738	0.024	0.532
Number of predacious taxa	-	-0.351	0.556	-1.620	**0.017**	-1.568	**0.088**	-1.035	0.368
Proportion stonerollers	+	-0.013	0.666	-0.022	0.497	0.011	0.801	0.101	**0.020**
Proportion centrarchid individuals	+	0.028	0.260	0.000	0.991	0.016	0.650	0.050	0.115
Number invert-eating taxa	-	-0.220	0.405	-0.514	**0.087**	-0.520	0.176	-0.352	0.490
Proportion invert-eating individuals	-	-0.005	0.866	-0.063	**0.094**	-0.059	0.244	-0.010	0.840
Number of benthic taxa	-	-0.297	**0.055**	-0.370	**0.026**	-0.349	0.119	0.019	0.960
Proportion benthic individuals	-	0.007	0.835	-0.041	0.210	-0.045	0.280	0.023	0.585
Number of lithophilic taxa	-	-0.325	0.327	-0.855	**0.002**	-1.208	**0.001**	-1.170	**0.020**
Proportion lithophilic individuals	-	-0.009	0.620	0.044	0.299	0.032	0.567	0.072	0.195

EXHIBIT R

Table 4.2. Percent change in fish indicators from minimum to maximum poultry house density according to models in Table 4.2.Min and Max are the values of indicators predicted by models in Table 4.2 with poultry house density set at minimum and maximum poultry house density. Umin and Umax are untransformed values for variables affected by transformations. %chng is the difference between metric values with PHD set and min and max divided by metric values with PHD set at minimum and then multiplying by 100 to get a percentage change.

Metric	Min	Max	Umin	Umax	% chng
Number of taxa	15.339	11.88			-22.5
Number of sensitive taxa	3.279	1.987			-39.4
Proportion sensitive individuals	0.331	0.166	0.109	0.028	-74.7
Proportion tolerant individuals	0.204	0.235	0.042	0.055	32.8
Number of predacious taxa	11.403	7.918			-30.6
Proportion stonerollers	0.198	0.222			12.4
Proportion centrarchid individuals	0.120	0.156			29.6
Number invert-eating taxa	4.971	3.815			-23.3
Proportion invert-eating individuals	0.343	0.212			-38.2
Number of benthic taxa	4.594	3.818			-16.9
Proportion benthic individuals	0.310	0.21			-32.3
Number of lithophilic taxa	9.784	7.098			-27.4

EXHIBIT R

Table 4.3. Percent change in fish indicators from minimum to maximum SMMNI (summer multimetric nutrient index) according to models in Table 4.2. Min and Max are the values of indicators predicted by models in Table 4.2 with SMMNI set at minimum and maximum values. Umin and Umax are untransformed values for variables affected by transformations. %chng is the difference between metric values with PHD set and min and max divided by metric values with PHD set at minimum and then multiplying by 100 to get a percentage change.

Metric	Min	Max	Umin	Umax	% chng
Number of taxa	16.43	11.82			-28.0
Number of sensitive taxa	3.283	2.221			-32.3
Proportion sensitive individuals	0.323	0.200	0.104	0.04	-61.9
Proportion tolerant individuals	0.185	0.238	0.034	0.057	64.3
Number of predacious taxa	12.39	7.932			-36.0
Proportion stonerollers	0.249	0.188			-24.3
Proportion centrarchid individuals	0.139	0.139			0.0
Number invert-eating taxa	5.285	3.871			-26.7
Proportion invert-eating individuals	0.383	0.210			-45.2
Number of benthic taxa	4.827	3.810			-21.1
Proportion benthic individuals	0.331	0.218			-34.1
Number of lithophilic taxa	9.839	7.488			-23.9

EXHIBIT R